Streptonigrin and Related Compounds. 6. Synthesis and Activity of Some Quinoxaline Analogues

Koppaka V. Rao* and Charles P. Rock

Department of Medicinal Chemistry, College of Pharmacy, Box J-485, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610

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Streptonigrin is an antitumor antibiotic with significant *in vitro* and *in vivo* antitumor activity spectrum. Besides the total syntheses, numerous syntheses of the partial structures have been carried out in order to assess the structural requirements for the activity. The present publication deals with the synthesis of selected quinoxalinequinones to study the effect of changes in the B-ring of streptonigrin.

A convenient 3-step synthesis of the required quinoxaline starting material that provides the necessary control of the desired substituent pattern is described. A series of 7-amino-6-methoxy-quinoxalinequinones were prepared in which substitution at 3- was varied (from H to Cl, OCH_3, CN, $COOCH_3$ and OH). A few selected 6-aminoquinones (with 6-NH_2 and 6-piperidinyl) were also prepared for comparison. The quinones (18) were tested in three different *in vitro* test systems: cytotoxicity to mouse leukemia (L-1210) cells, antibacterial activity against *Bacillus subtilis* and inhibition of root growth. Several of the amino-quinones showed significant activity in comparison to that shown by streptonigrin.

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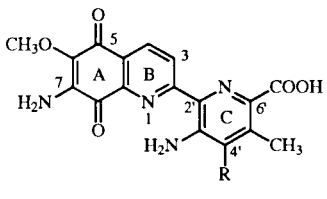
Introduction.

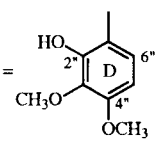
The antitumor antibiotic, streptonigrin **1a** isolated from *Streptomyces flocculus* [1] in 1959, has a wide range of activity covering a variety of transplanted tumors [2-4] and viruses including HIV-1 [5,6]. In human trials, streptonigrin was active against malignant lymphomas and chronic lymphoblastic leukemia and others, singly [7,8] as well as in combination-therapy [9,10]. In the presence of O_2, NADH, and a cation, *e.g.* Cu^{+2} or Fe^{+2}, **1a** generates a semi-quinone or a $OH^{\bullet}$ and thus causes single strand breaks in DNA[11,12]. It inhibits topoisomerase II [12] and reverse transcriptase [13]. Complexes, readily formed between **1a** and certain divalent transition metal cations, bind to DNA [14].

The unique tetracyclic structure **1a** [15,16] of streptonigrin, has prompted numerous syntheses of simpler quinoline quinones [17,18], fully substituted tricyclic system **1b** [19] and finally, three total syntheses [20-22]. By the use of different bioassays, it was found that different partial structures retained different degrees of activity of **1a.**

A series of simpler tricyclic analogues were also prepared and tested, in which ring A had the 7-amino-6-methoxy or the alternative 6-amino-7-methoxyquinone system; with ring B, as in quinoline or isoquinoline and ring C, a phenyl or a 2,2-pyridyl group, clearly showed that an aminoquinone was necessary for activity, and this may be modulated by variations in the other rings [17,23,24]. However, data on the changes in ring B are scarce, other than those on the 1-phenyl and 1-benzylisoquinoline-5,8-diones, which clearly raised some questions on the generally accepted structural requirements for activity [23].



1a, R =



1b, R = H

Present Target Compounds and Rationale for their Selection.

To pursue the effects of changes in ring B further, synthesis and testing of some quinoxalinequinone analogues of **1a** was undertaken. Thus, compounds with ring A being the same as that in **1a**; ring B, a pyrazine ring where a number of substituents may be introduced; and ring C as a phenyl group, are targeted. Testing these in the same *in vitro* assays as used before, will permit comparisons with the quinoline/isoquinoline analogues as well as with **1a**.

The idea that replacing the quinoline ring by a quinoxaline ring might reflect in the activity was based on the properties of the two systems, some similar and some dissimilar, *e.g.* the widely different pK_as for protonation: 4.96 and 0.6 for the quinoline-*N* and the quinoxaline-N_1, respectively [25,26]. Positions 2 and 3 of quinoxaline and 2 and 4 of quinoline, with a low electron density, are amenable for reactions such as reductions, Chichibabin and Reissert reactions. The results from electrophilic reactions are also alike. However, the reduction potentials of the respective 5,8-diones differ, decreasing in the order: phthalazine > quinazoline > quinoxaline > isoquinoline > quinoline > naphthalene [27].

With regard to ring C, the choice of a phenyl group over a 2-pyridyl group substituted at the 2-position of the quinoxaline ring was made for this study, because the activities of the analogues with either group were comparable [19,23].

Chemistry.

The well-known condensation of a *o*-phenylenediamine with an α,β-dicarbonyl compound for the synthesis of a quinoxaline ring is useful only when there is symmetry in either of the starting materials; otherwise, a mixture of isomers will result. Thus, the need for specified substituents at positions 2, 3, 6, and 7 in this study, precludes the use of this method in favor of the following method.

Synthesis of the Lead Compound. Outline of Further Steps.

As shown in Scheme 1, *N*-(3,5-dimethoxyphenyl)benzoylformamide (**2**) was nitrated to give a 4:1 mixture of the 2- and the 4-nitro derivatives **3** and **4** which on reduction, gave the 2(1*H*)-quinoxalinones **5**, and **6** respectively. Halogenation of **5** to **7** (or **8**) and reduction gave the lead compound 6,8-dimethoxy-2-phenylquinoxaline **9**. This route gave good overall yields, with the needed positional control of the substituents.

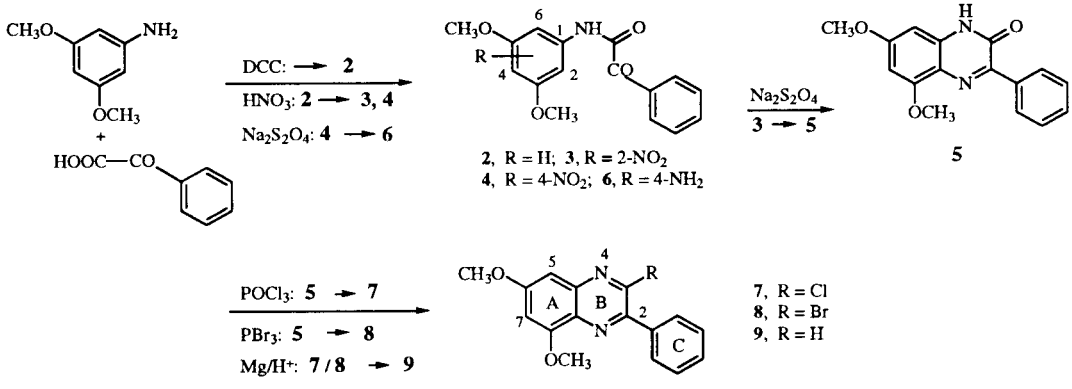
Using quinoxalines **5**, **7**, **8** and **9** generated as above, others containing different 3-substituents, *e.g.* 3-OCH_3, 3-CN, 3-$COOCH_3$, were prepared, as well as the *N*-methyl-2(1*H*)-quinoxalinone. Each of these was then converted to the corresponding 7-amino-6-methoxyquinone.

From the 3-chloro and the 3-cyanoquinoxalines, the alternative 6-aminoquinones, as well as some 3- and/or 6-piperidinoquinones were prepared for this study.

7-Aminoquinoxalinequinones.

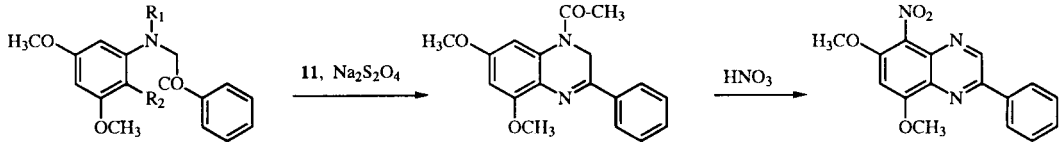
For the conversion of the above quinoxalines to the target 7-amino-6-methoxyquinones two general routes were used (Scheme 3). Thus, under conditions of nitration, the 6,8-dimethoxyquinoxalines gave mainly the 5-nitro compounds, with varying amounts of the 5,7-dinitro-8-hydroxyquinoxalines (the dinitrophenols). In the first route (a), the 5-nitro derivatives were reduced to the amines, where possible (*i.e.*, without the loss of the 3-substituent) and then oxidized to the 5,8-diones. In some cases, the nitration itself gave the 5,8-diones directly. Amination of the quinone was carried out by reaction with sodium azide, as was described before [17] or, as in some cases, by the standard scheme of bromination (at 7), replacement by an azide and reduction. The second and preferred route (b) to the quinones consisted of reduction of the dinitrophenols to the diaminophenols, which underwent air-oxidation to give the desired 7-amino-6-methoxyquinones.

Scheme 1: Synthesis of the Lead Compounds



An alternative approach was also studied, with a view to generate analogues with a dihydroquinoxaline ring, as shown in Scheme 2, consisting of alkylation of 3,5-dimethoxyaniline with 2-bromoacetophenone to give **10**, acetylation/nitration to provide **11** and reductive cyclization of **11** to the dihydroquinoxaline acetate **12**. Nitration of **12** gave, however, the 5-nitroquinoxaline **13**, instead of the expected dihydroquinoxaline, and thus this method served as an alternative approach for **13**.

Scheme 2: Alternative Synthesis of the Lead Compound

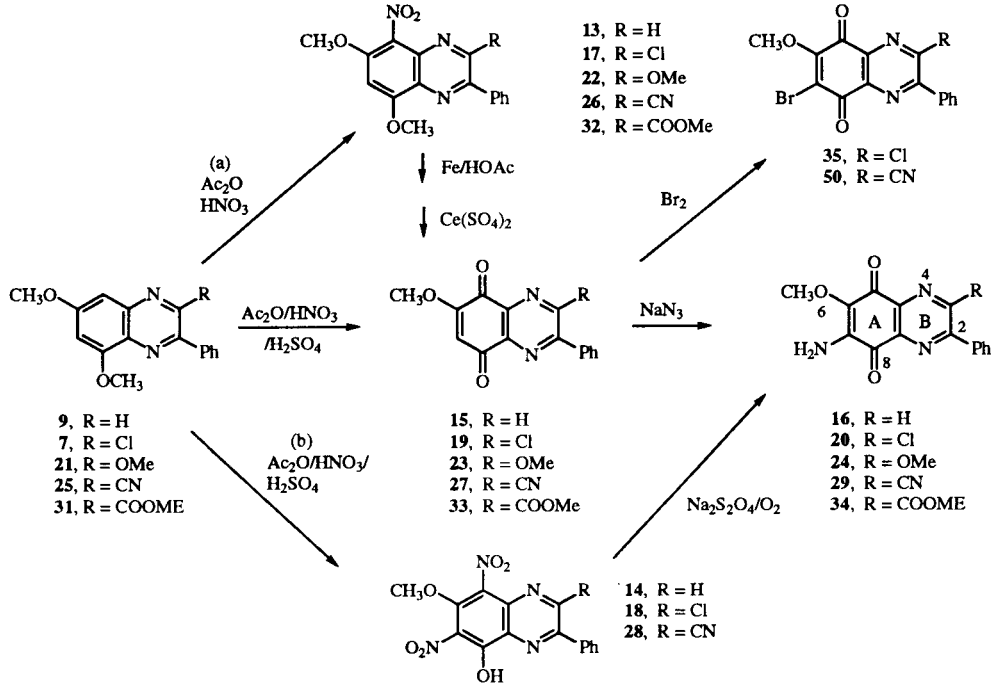


10, $R_1 = R_2 = H$
11, $R_1 = CO\text{-}CH_3$, $R_2 = NO_2$

12

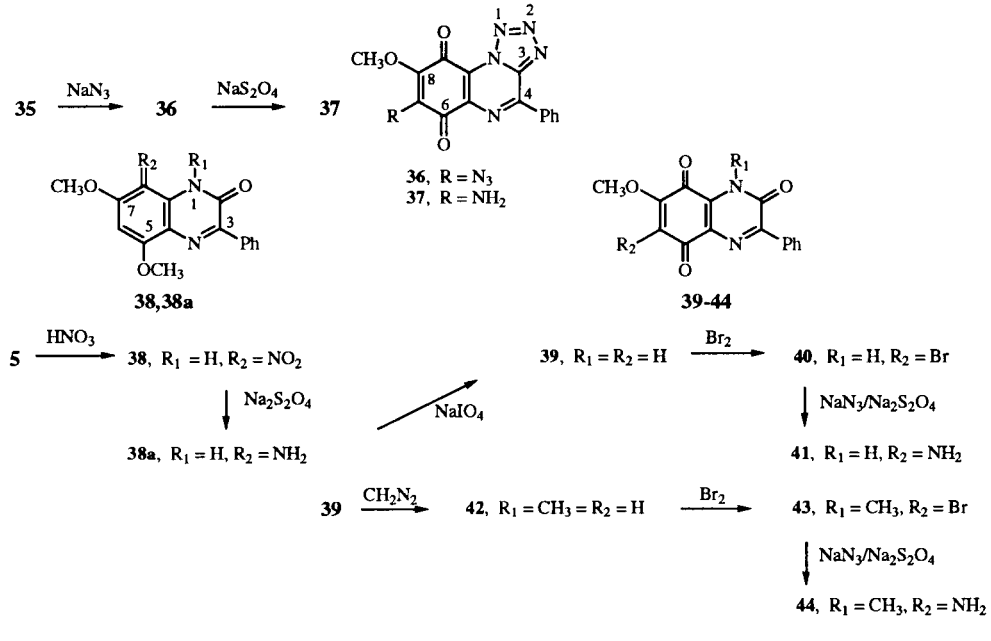
13

Scheme 3: Methods for the Generation of the 7-Aminoquinoxalinequinones



By the use of routes a) and/or b) outlined above, most of the 7-aminoquinones shown in Scheme 3 could be readily prepared from the appropriate quinoxaline with the 3-H (**9**), 3-OMe (**21**), and the 3-COOMe (**31**) substituents. However, some changes to the general approach had to be made with the 3-Cl and the 3-CN derivatives. First, the 3-chloro-5-nitro derivative **17** could not be reduced without dehalogenation occurring simultaneously. To avoid this, further nitration of **7** was tried to prepare the dinitrophenol **18** as the major product, but it gave, unexpectedly, the 5,8-dione **19** as the major product, with some **18** (Scheme 3). Direct amination of **19** with sodium azide also failed, and hence the alternative azide route was studied using the 7-bromoquinone **35**. Here also, the azide displaced both the 7-bromo and the 3-chloro substituents to form the 7-azidotetrazoloquinolinequinone **36**, from

Scheme 4: Additional 7-Aminoquinoxalinequinones



which the 7-aminotetrazoloquinoxalinequinone **37** was prepared (Scheme 4).

Reduction of the 3-cyano-5-nitro derivative **26** also led, unexpectedly, to the loss of the cyano group. Further nitration of **26** gave the 5,8-dione as a significant product as seen with the 3-chloro derivative.

For the 2(1*H*)-quinoxalinones, the dinitrophenol pathway could not be used because nitration attacked the 3-phenyl ring. The target aminoquinones **41** and **44** (Scheme 4) were, however, prepared from the 5,8-diones **39** and **42** *via* the 7-bromo and the 7-azido derivatives (Scheme 4).

The quinone **39** (Scheme 4) was found to be strongly acidic and methylation (using diazomethane) gave essentially a single product, different from **23** (seen in Scheme 3), and hence formulated as the *N*-methyl form **42**, despite its nmr signal for its N-Me appearing at 3.9 ppm.

6-Aminoquinoxaline-5-8-diones.

The ready displacement of the 3-chloro group in **35** by the azide (as seen in Scheme 4) prompted a brief study into the reactivity of the 3-chloro group in comparison with the 6, and 7 positions of the quinone ring, using **35** and **19**, along with the non-quinonoid **7**, with piperidine and ammonia as the nucleophiles.

Although **7** reacted with piperidine at 100° to give **45**, **19** formed **46** even at 0-5° (Scheme 5). With **35**, under the same conditions, displacement of the 6-methoxyl took place, instead, to give **47**. A similar displacement was reported by Pratt in the case of 6-methoxy-7-bromoquinolinequinone[29]. The presence of bromine at 7 (in **35**), evidently enhances the reactivity of the C-6 over that of the C-3. However, with an excess of piperidine, both **19** and **35** gave the 3,6-dipipendino derivatives **48** and **49**.

With ammonia as the nucleophile, **35** and **50** readily yielded the corresponding 6-amino-7-bromoquinones **51** and **52** (Scheme 5).

Biological Activity.

The eighteen quinoxalinequinones described here were tested in three different *in vitro* assay systems: 1) cytotoxicity to mouse leukemia (L1210) cells, 2) antibacterial activity against *Bacillus subtilis* and 3) the inhibition of root growth using seedlings of *Lepidium sativum*.

Of the eighteen quinones tested, eight are the 7-amino-6-methoxyquinones (similar to **1a**), six are 6-aminoquinones (with amino or piperidinyl group) and the other four are non-aminoquinones used for comparison. Results of comparison with **1a** and with the previously known and tested 6-amino-6-methoxy-2-phenylquinoline-5,8-dione **53** (Scheme 5), whose activity has been correlated with that of **1a** are presented.

Results and Discussion.

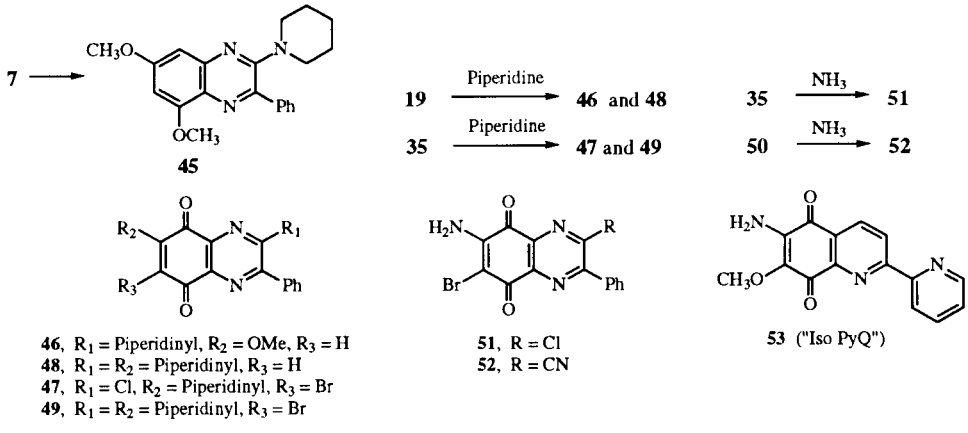
L-1210 Cytotoxicity.

As seen in Table 1, most of the 7-amino-6-methoxyquinones with the exception of **41**, displayed significant activity which were 1-5 times that of the reference, Iso-PyQ **53**, and show a relative potency (RP) of 1/5 to 1/50 that of **1a**. The most active of this group were **20** (RP-1/5) and **29** (RP-1/12). The lowest activity was shown by **41**, with the possible explanation that its highly polar nature might have prevented the entry into the cell. The corresponding *N*-methyl derivative **44** was much more active.

The most active compounds appeared to be the 6-amino-7-bromoquinones, **51** and **52**, (RP of 2/5), thereby suggesting that the amino group can be at either the 6 or 7 in the quinone ring. The quinoxaline analogues with the 6-amino group were also considerably more active than the corresponding quinoline analogue **53**, which also had the 6-amino group. Thus, further studies along these lines, *i.e.*, using a quinoxaline as the B-ring, with the 6- and 7-amino substitution and replacing the methoxyl with a halogen, will be justified.

All of the piperidinoquinones showed a similar range of activity, with the best being the 3-piperidinyl-6-methoxyquinoxalinequinone **46**, although it does not have the R-NH group in ring A. Further work with other basic substituents at 3 may be promising. In general, it appears that a simple amino group at either the 6 or 7 position imparts

Scheme 5: Preparation of 6-Aminoquinoxalinequinones



46, R_1 = Piperidinyl, R_2 = OMe, R_3 = H
48, $R_1 = R_2$ = Piperidinyl, R_3 = H
47, R_1 = Cl, R_2 = Piperidinyl, R_3 = Br
49, $R_1 = R_2$ = Piperidinyl, R_3 = Br

51, R = Cl
52, R = CN

Table 1
Activity of the Quinoxalinequinones

Compound No.	L-1210 IC50			*B. subtilis* MIC			Root Growth IC50		
	ng/ml	n*M*	Relative potency	ng/ml	n*M*	Relative potency	ng/ml	n*M*	Relative potency
16	43	0.15	0.065	0.8	3	0.46	4.45	16	0.8
20	16	0.05	0.196	0.8	2.5	0.55	9.45	30	0.42
37	140	0.43	0.023	22	68.3	0.02	>20	–	–
24	125	0.4	0.025	1.1	3.4	0.4	12.9	41	0.31
29	39	0.13	0.079	0.9	2.9	0.47	3.8	12	1
34	57	0.17	0.06	1.1	3.2	0.435	10.5	31	0.41
41	1242	4.18	0.004	>20	>100	–	9.4	32	0.4
43	176	0.49	0.021	16	45	0.031	83	832	0.06
44	127	0.41	0.025	0.7	2.4	0.58	9.9	32	0.4
46	41	0.12	0.086	4	11.4	0.12	11.5	33	0.38
47	107	0.25	0.04	>20	–	–	>20	–	–
48	193	0.48	0.021	100	–	–	>20	–	–
49	165	0.34	0.029	0.8	1.7	0.8	>20	–	–
51	9	0.02	0.417	1	2.7	0.51	10.5	29	0.44
52	10	0.03	0.37	0.8	2.3	0.61	7.4	21	0.61
1a	5	0.01	1	0.69	1.4	1	6.4	12.6	1
53	139	0.5	0.02	1	3.7	0.37	1.26	5	2.8
19	247	0.82	0.012	39.8	132	0.01	–	–	–
23	382	1.29	0.008	19.8	66.9	0.021	18	61	0.21
39	1609	5.7	0.002	43	152	0.009	–	–	–

better activity than a substituted amino group. Among the non-aminoquinones, the 7-bromo-6-methoxy-2(1*H*)-quinoxalinonequinone **40** showed activity comparable to that of the 7-amino-6-methoxyquinones, again showing the positive influence of a halogen on the activity. The low degree of activity of the rest of these, however, reinforces the generally accepted view that an aminoquinone is necessary for the best activity.

Antibacterial Assay.

Most of the compounds showed good activity in this test as seen in Table 1, although in a few cases, problems with precipitation and/or lack of diffusion, led to results not acceptable for evaluation. Unlike what was seen with the cytotoxic activity, where the relative potencies (RP) are in the range of 2-40%, the values here are in a much higher range: 40-80%, thus showing the different structural requirements for the different activities and hence the need for different types of tests in drawing valid conclusions.

Most of the 7-amino-6-methoxyquinones showed good activity, with the exception of the tetrazoloquinone **37**. The 6-amino- and the piperidinoquinones were highly active, in some cases, surpassing the 7-aminoquinones in their activity. The non-aminoquinones, in general, showed only a low degree of activity.

The quinoxaline analogues seem to be almost as active as the quinoline analogue **53** chosen here for comparison, whereas they appear to be 5-10 times more active than the isoquinoline analogues based on the published data [23].

Root-growth Inhibition.

This assay is said to reflect the effect of the drug on the DNA synthesis [32]. Once again, it can be seen that there are yet, different structural requirements for activity in this system. For example, the isoPyQ **53** is nearly 3 times as active as **1a**, although in the cytotoxicity assay, it was only 1/50 as active.

The IC_{50} values for the quinoxaline analogues are presented in Table 1 along with those of streptonigrin and IsoPyQ. In this test, it is evident that the quinoxaline analogues are generally weaker (10-30%) in activity compared to the IsoPyQ **53** and the isoquinoline analogues reported [23].

Most of the 7-amino-6-methoxyquinoxalinequinones were active, with the exception of the tetrazoloquinone **37**. The most active were the 3-H and 3-CN quinones. Although the 6-amino-7-bromoquinones were comparable in activity to the 7-aminoquinones, the piperidinoquinones showed only weak activity. The non-aminoquinones were relatively inactive, although there was no diffusion problem with these in this test system.

A comparison of the activities of selected compounds which showed significant activity in all three assays may be seen in the bar graph, Figure 1.

Thus, somewhat as expected, the quinoxalinequinones with an amino function at either the 6 or 7 positions show significant activity in all three test systems. It is quite encouraging to note that simple aminoquinones such as **20**, **51** and **52** can reach a level of 20-40% of the cytotoxi-

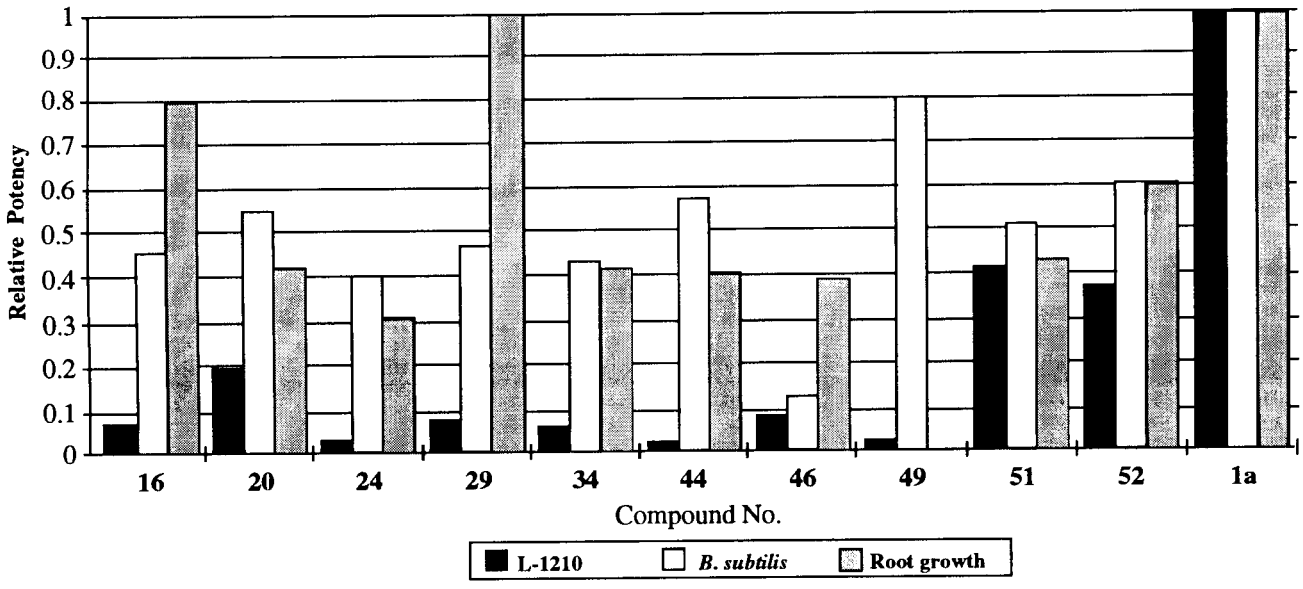


Figure 1. Acitivities of Selected Quinoxalinequinones.

city, 40-60% of the antibiotic activity and 40-60% of the root growth inhibiting activity shown by a complex molecule such as streptonigrin. Unlike the quinoline and isoquinoline analogues which were much more active in one test compared to another (*e.g.* isoPyQ **53** is 3 times as active in the root growth inhibition assay, but only 2% as active as streptonigrin in the cytotoxicity assay), the quinoxaline quinones appear to be active more uniformly in all three tests as seen from Figure 1.

Using the quinoxalinequinone system, further variations involving halogens at the 6 and 7 positions, substitution at the 3-position, changes in ring C, all appear to be promising for further study.

EXPERIMENTAL

General.

Melting points were determined using the Fisher-Johns hot stage apparatus and are uncorrected. The following instruments were used for the spectra recorded here: ir, Beckman Acculab III, as potassium bromide pellets; nmr, Varian EM 390, with the chemical shifts being reported in parts per million (δ) relative to tetramethylsilane used as the internal standard, and mass spectra, MS80RFA (Kratos Analytical) and FAB11NF (Ion Tech. Ltd). Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA.

Column chromatography was carried out using silica gel (Fisher 100-200 mesh) and thin-layer chromatography with silica gel HF-60, 254+366 (EM Science, Fisher).

N-(3,5-Dimethoxyphenyl)benzoylformamide **2**.

To a mixture of benzoyl formic acid (14.5 g) and 3,5-dimethoxyaniline (14.5 g) in 1:1 acetone/benzene (200 ml) at 0° was added dicyclohexylcarbodiimide (21 g) in benzene (100 ml) over 3 minutes. After 15 minutes of stirring at 0° and 15 minutes at 20-25°, water (150 ml) was added, the mixture stirred for 1 hour and then filtered. The aqueous layer of the filtrate was extracted with fresh benzene. The precipitate was extracted with chloroform until most of the color was extracted, leaving dicyclohexylurea. The combined solvent extract was concentrated to dryness and the solid crystallized from 9:1 ether/methanol to give **2** as yellow needles, yield 22.9 g (85%), mp 126-128°; ^{1}H nmr (deuteriochloroform): 8.80 (1 H, br, NH), 8.27-8.38 (2 ArH, m), 7.45 (3 ArH, m), 6.88 (2 H, d, J = 2 Hz), 6.27 (1 H, dd, J = 2 Hz), 3.73 (6 H, s); ir: 3345, 2935, 1675, 1650, 1585 cm^{-1}.

Anal. Calcd. for $C_{16}H_{15}NO_4$: C, 67.35; H, 5.30; N, 4.91. Found: C, 67.28; H, 5.34; N, 4.95.

N-(3,5-Dimethoxy-2-nitrophenyl)benzoylformamide **3**.

To a suspension of **2** (9 g) in acetic anhydride (40 ml) at 0-5°, was added 70% nitric acid (7 ml) drop-wise with stirring over 5 minutes and the stirring continued for another 30 minutes. After the addition of ice/water and stirring for 1 hour, the precipitate was filtered, dissolved in chloroform, and washed with aqueous sodium bicarbonate. The chloroform layer was concentrated to dryness and the solid **3**, crystallized from ether, yield 7.4 g (71%), mp 163-165°; ^{1}H nmr (deuteriochloroform): 10.73 (1 H. br), 8.28-8.37 (2 H, m), 7.77 (1 H, d, J = 3 Hz), 7.40-7.63 (3 H, m), 6.40 (1 H, d, J = 3 Hz), 3.90 (6 H, s); ir: 3315, 3115, 2940, 2920, 1710, 1680 cm^{-1}.

Anal. Calcd. for $C_{16}H_{14}N_2O_6$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.08; H, 4.24; N, 8.48.

N-(3,5-Dimethoxy-4-nitrophenyl)benzoylformamide **4**.

The filtrates of **3** were chromatographed (silica/benzene). The product crystallized as orange needles (2:1 ether/ligroin), mp 158-160°; ^{1}H nmr (deuteriochloroform): 9.03 (1 H, br), 8.30-8.37 (2 H, m), 7.33-7.77 (3 H, m), 7.05 (2 H, s), 3.87 (6 H, s); ir: 3345, 3075, 2980, 2940, 1700, 1675 cm^{-1}.

Anal. Calcd. for $C_{16}H_{14}N_2O_6$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.10; H, 4.24; N, 8.48.

5,7-Dimethoxy-3-phenylquinoxalin-2(1*H*)-one **5**.

A mixture of **3** (17 g) and sodium dithionite (45 g, 5 equivalents) in methanol/water (1:4, 500 ml) was boiled under reflux for 30 minutes. The cooled mixture was diluted with water (200 ml), the solid filtered and washed, first with water and then with ether, to give **5** (8.2 g) as light yellow crystals (ether/ligroin). From the filtrate, another 1.73 g of **5** was recovered, yield 9.93 g (72%), mp 256-258°; uv: maxima 365 nm (major), 265 nm (minor); ^{1}H nmr (deuteriochloroform/DMSO-d_6): 12.12 (1 H, br), 8.19-8.27 (2 H, m), 7.31-7.38 (3 H, m), 6.25 (2 H, 2d, J = 2 Hz), 3.95 (3 H, s), 3.80 (3 H, s); ir: 3450, 3100, 2940, 1645, 1620 cm^{-1}.

Anal. Calcd. for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92. Found: C, 68.02; H, 5.00; N, 9.94.

N-(4-Amino-3,5-dimethoxyphenyl)benzoylformamide **6**.

The filtrates from **5** after chromatography (silica/benzene) gave **6** as orange crystals (ether), yield 1.79 g (13%), mp 141-143°; ^{1}H nmr (deuteriochloroform): 8.70-8.87 (1 H, br), 8.30-8.40 (2 H, Ar m), 7.30-7.63 (3 H, m), 6.93 (2 H, s), 3.83 (6 H, s), 3.50-4.00 (2 H, br); ir: 3420, 3340, 3000, 2960, 1680, 1670, 1625 cm^{-1}.

Anal. Calcd. for $C_{16}H_{14}N_2O_4$: C, 63.99; H, 5.37; N, 9.33. Found: C, 64.07; H, 5.36; N, 9.26.

3-Chloro-6,8-dimethoxy-2-phenylquinoxaline **7**.

A mixture of **5** (6 g) and phosphorus oxychloride (10 ml) was heated at reflux for 30 minutes. After cooling to (0°), ice was added (stirring) and after 30 minutes the solid was filtered, and crystallized (ether) to give **7** as off-white crystals, yield 6.0 g (94%), mp 188-190°; uv: maxima: 365 nm (minor), 265 nm (major); ^{1}H nmr (deuteriochloroform): 7.70-7.78 (2 H, m), 7.37-7.43 (3 H, m), 6.88 (1 H, d, J = 2.5 Hz), 6.83 (1 H, d, J = 2.5 Hz), 3.97 (3 H, s), 3.90 (3 H, s); ir: 3060, 2940, 1615 cm^{-1}.

Anal. Calcd. for $C_{16}H_{13}N_2O_2Cl$: C, 63.90; H, 4.36; N, 9.31. Found: C, 64.14; H, 4.33; N, 9.13.

3-Bromo-6,8-dimethoxy-2-phenylquinoxaline **8**.

A mixture of phosphorus tribromide (10 ml) and **5** (0.98 g) was stirred at 135° (oil bath) for 1.25 hours. It was chilled and stirred with ice at 0-5° for 0.5 hours. The solid was filtered and crystallized from ether, yield 0.62 g (50%), mp 193-194°; ^{1}H nmr (deuteriochloroform): 7.63-7.83 (2 H, m), 7.33-7.57 (3 H, m), 6.97 (1 H, d, J = 2 Hz), 6.73 (1 H, d, J = 2 Hz), 4.02 (3 H, s), 3.95 (3 H, s); ir: 3050, 3000, 2960, 2915, 2820, 1615 cm^{-1}.

Anal. Calcd. for $C_{16}H_{13}N_2O_2Br$: C, 55.67; H, 3.80; N, 8.11. Found: C, 55.79; H, 3.86; N, 8.20.

6,8-Dimethoxy-2-phenylquinoxaline **9**.

A solution of **7** (0.98 g) in acetic acid (6 ml) was stirred with magnesium turnings (0.9 g) at 85° for 30 minutes. Water was added, and the mixture extracted with chloroform. The solid from the extract was crystallized (9:1 ether/acetone) to give **9** as a light-brown crystalline solid, yield 0.72 g (85%), mp 138-140°; uv: maxima 278 nm (major); ^{1}H nmr (deuteriochloroform): 9.15 (1 H, s), 8.07-8.13 (2 H, m), 7.43-7.50 (3 H, m), 7.00 (1 H, d, J = 2 Hz), 6.74 (1 H, d, J = 2 Hz), 3.87 (3 H, s), 3.77 (3 H, s); ir: 3060, 3000, 2940, 2835, 1610 cm^{-1}.

Anal. Calcd. for $C_{16}H_{14}N_2O_2$: C, 72.16; H, 5.30; N, 10.52. Found: C, 71.90; H, 5.27; N, 10.47.

N-(3,5-Dimethoxyphenyl)-α-benzoylmethylamine **10**.

A mixture of 3,5-dimethoxyaniline (1 g) and sodium bicarbonate (1 g) in dimethylformamide (12 ml) was stirred with α-bromoacetophenone (1.3 g) at 50° for 2 hours. After dilution with water (100 ml), the solid was filtered and crystallized from ether to give **10** as pale-yellow crystals, yield 1.55 g (88%), mp 112-115°; ^{1}H nmr (deuteriochloroform): 7.77-8.00 (2 H, m), 7.27-7.60 (3 H, m), 5.87 (3 H, s), 4.53 (2 H, s), 3.73 (6 H, s), 3.1-3.5 (1 H, br); ir: 3380, 3060, 3000, 2955, 2835, 1690, 1620, 1580 cm^{-1}.

Anal. Calcd. for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16. Found: C, 70.71; H, 6.30; N, 5.09.

N-Benzoylmethyl(3,5-dimethoxy-2-nitrophenyl)acetamide **11**.

A solution of **10** (3.65 g) in acetic anhydride (10 ml) was heated at 80° for 30 minutes. To the cooled (5°) mixture was added nitric acid (3.0 ml) drop-wise with stirring, and ice after 30 minutes. The solid was chromatographed (silica/benzene) and the major product crystallized from ether/ligroin, yield 3.47 g (72%); mp 113-115°; ^{1}H nmr (deuteriochloroform): 7.73-7.93 (2 H, m), 7.27-7.57 (3 H, m), 6.90 (1 H, d, J = 2 Hz), 6.53 (1 H, d, J = 2 Hz), 5.63 (1 H, d, J = 18 Hz), 4.27 (1 H, d, J = 18 Hz), 3.85 (3 H, s), 3.82 (3 H, s), 2.00 (3 H, s).

Anal. Calcd. for $C_{18}H_{18}N_2O_6$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.30; H, 5.05; N, 7.81.

4-Acetyl-6,8-dimethoxy-2-phenyl-3,4-dihydroquinoxaline **12**.

A solution of **11** (3.0 g) in methanol (15 ml) was boiled with sodium dithionite (7 g) in water (40 ml) for 30 minutes. After dilution with water (100 ml), the solid was crystallized from ether to form white crystals, yield 1.7 g (65%); ^{1}H nmr (deuteriochloroform): 7.77-8.03 (2 H, m), 7.20-7.53 (3 H, m), 6.40 (2 H, br), 4.70 (2 H, s), 3.93 (3 H, s), 3.83 (3 H, s), 2.27 (3 H, S).

Anal. Calcd. for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.80; H, 5.73; N, 9.11.

6,8-Dimethoxy-5-nitro-2-phenylquinoxaline **13**.

To a stirred suspension of **9** (0.98 g) in acetic anhydride (5 ml) was added nitric acid (70%, 0.8 ml) drop-wise at 22°. When tlc showed the absence of **9**, ice was added, the solid filtered, dissolved in chloroform, washed with aqueous sodium bicarbonate and the chloroform concentrated to dryness. The solid was crystallized from acetone/ligroin to give **13** as yellow crystals, yield 0.72 g (63%), mp 267-270°; ^{1}H nmr (deuteriochloroform/DMSO-d_6): 9.33 (1 H, s), 8.12-8.20 (2 H, m), 7.08-7.23 (3 H, m), 4.20 (3 H, s), 4.14 (3 H, s); ir: 3060, 3020, 2980, 2950, 2850, 1620, 1580 cm^{-1}; ms: Calcd. for $C_{16}H_{13}N_3O_4$, 311. Found 311.

5,7-Dinitro-8-hydroxy-6-methoxy-2-phenylquinoxaline **14**.

The reaction given under **13** was kept until tlc showed the absence of **13** and the presence of a yellow spot at the origin. After work up as before, the solid was dissolved in chloroform, and washed with aqueous sodium bicarbonate. The aqueous layer was acidified and filtered. Crystallization from ether gave **14**, yield 0.12 g (75%), mp 218-220°; ^{1}H nmr (deuteriochloroform): 9.40 (1 H, s), 8.26-8.33 (2 H, m), 7.45-7.53 (3 H, m), 4.70 (1 H, br), 4.07 (3 H, s); ir: 3360, 2950, 1630, 1580 cm^{-1}.

Anal. Calcd. for $C_{15}H_{10}N_4O_6$: C, 52.64; H, 2.95; N, 16.37. Found: 52.60; H, 2.98; N, 16.42.

6-Methoxy-2-phenylquinoxaline-5,8-dione **15**.

A solution of **13** (0.3 g) in acetic acid (5 ml) was heated with reduced iron (0.2 g) at 85° for 10 minutes. After dilution with water (100 ml), the mixture was extracted twice with chloroform (2 x 70 ml). The chloroform extract was stirred with 2*N* sulfuric acid containing 5% ceric sulfate. The chloroform layer was separated, washed with water, concentrated to dryness and the product crystallized from acetone/ether, yield 0.198 g (77%), mp 180-183°; ^{1}H nmr (deuteriochloroform): 9.30 (1 H, s), 8.00-8.20 (2 H, m), 7.33-7.57 (3 H, m), 6.40 (1 H, s), 3.93 (3 H, s); ir: 3045, 2915, 2840, 1690, 1660, 1600, 1560 cm^{-1}.

Anal. Calcd. for $C_{16}H_{13}N_3O_4$: C, 61.73; H, 4.21; N, 13.50. Found: C, 61.55; H, 4.10; N, 13.33.

7-Amino-6-methoxy-2-phenylquinoxaline-5,8-dione **16**.

Method A.

A mixture of **14** (0.1 g) in methanol (3 ml), 5% aqueous sodium bicarbonate (3 ml) and sodium dithionite (0.6 g) was heated at reflux until the starting material was absent. It was acidified (*p*H 4) and extracted with chloroform. The chloroform was shaken with water containing 5% ceric sulfate in 2*M* sulfuric acid (4 ml). The reddish-brown solvent layer was concentrated to dryness, and the residue crystallized from 2:1 acetone/ligroin to give **16** as a dark-brown crystalline solid, yield 0.027 g (35%), mp 268-270° dec; ^{1}H nmr (deuteriochloroform/DMSO-d_6): 9.33 (1 H, s), 8.03-8.30 (2 H, m), 7.33-7.67 (3 H, m), 6.70 (2 H, br), 3.93 (3 H, s); ir: 3485, 3260, 3100, 2960, 1690, 1645, 1590, 1580 cm^{-1}.

Anal. Calcd. for $C_{15}H_{11}N_3O_3$: C, 64.05; H, 3.94; N, 14.94. Found: C, 64.23; H, 4.08; N, 14.72.

Method B.

A solution of **13** (0.094 g) in acetic acid (5 ml) was heated with sodium azide (0.1 g) at 55° with stirring for 1 hour. When the starting material was absent, the mixture was diluted with water and extracted with chloroform. Chromatography (silica with 1:1 chloroform/benzene) gave **16**, which was crystallized from 2:1 acetone/ligroin, yield 0.015 g (15%). Physical and spectral data showed that this was identical to the one obtained from the dinitrophenol **14**.

3-Chloro-6,8-dimethoxy-5-nitro-2-phenylquinoxaline **17**.

The procedure given under **13** was used with **7** (1.27 g), acetic anhydride (6 ml) and nitric acid (70%, 1.5 ml), yield of **17** (acetone), 1.17 g (74%), mp 212-214°; ^{1}H nmr (deuteriochloroform/DMSO-d_6): 7.68-7.78 (2 H, m), 7.45-7.52 (3 H m), 6.80 (1 H, s), 4.10 (6 H, s); ir: 2970, 1615 cm^{-1}.

Anal. Calcd. for $C_{16}H_{12}N_3O_4Cl$: C, 55.60; H, 3.50; N, 12.16. Found: C, 55.67; H, 3.50; N, 12.16.

3-Chloro-6-methoxy-2-phenylquinoxaline-5,8-dione **19**.

A solution of **7** (1.0 g) in chloroform (6 ml) and acetic anhydride (8 ml) was stirred with nitric acid (70%, 0.8 ml), at 20° until **7** was absent (tlc). A 1:1 mixture of sulfuric acid/nitric acid (0.4 ml) was added, followed after 10 minutes, by ice. The filtered solid was partitioned between chloroform and aqueous sodium bicarbonate. The solid from chloroform was crystallized from ether, yield 0.4 g (40%), mp 262-264°; ^{1}H nmr (deuteriochloroform): 7.92-7.83 (2 H, m), 7.50-7.58 (3 H, m), 6.40 (1 H, s), 3.97 (3 H, s); ir: 3050, 3020, 2945, 1700, 1666, 1610 cm^{-1}.

Anal. Calcd. for $C_{15}H_9N_2C_3Cl$: C, 59.91; H, 3.02; N, 9.32. Found: C, 59.94; H, 3.01; N, 9.32.

3-Chloro-5,7-dinitro-8-hydroxy-6-methoxy-2-phenylquinoxaline **18**.

The sodium bicarbonate washings from **19** were acidified, the solid chromatographed (silica/benzene) to give **18** as a crystalline solid (ehter/ligroin), 0.11 g (9%), mp 186-187°; ^{1}H nmr (deuteriochloroform): 7.78-7.85 (2 H, m), 7.53-7.60 (3 H, m), 4.13 (3 H, s), 3.75 (1 H, br); ir: 3380, 3065, 3020, 2940, 1640 cm^{-1}.

The monoacetate of **18** was prepared (acetic anhydride, 100° 15 minutes) and crystallized from ether, mp 173-175°; ^{1}H nmr (deuteriochloroform): 7.75-7.85 (2 H, m), 7.50-7.57 (3 H, m), 4.17 (3 H, s), 2.43 (3 H, s); ir: 2960, 2940, 1800, 1625 cm^{-1}.

Anal. Calcd. for $C_{17}H_{11}N_4O_7Cl$: C, 48.76; H, 2.65; N, 13.38. Found: C, 49.01; H, 2.70; N, 13.27.

7-Amino-3-chloro-6-methoxy-2-phenylquinoxaline-5,8-dione **20**.

The procedure given under **16** was used with **18** (0.1 g), and sodium dithionite (0.6 g). The product **20** was crystallized from acetone, yield 0.029 g (35%), mp 270-275° dec; ^{1}H nmr (deuteriochloroform): 7.67-7.83 (2 H, m), 7.33-7.53 (3 H, m), 6.00-6.25 (2 H, br), 4.00 (3 H, s); ir: 3470, 3340, 2950, 1685 cm^{-1}.

Anal. Calcd. for $C_{15}H_{10}N_3O_3Cl$: C, 57.06; H, 3.20; N, 13.31. Found: C, 56.89; H, 3.36; N, 13.12.

2-Phenyl-3,6,8-trimethoxyquinoxaline **21**.

A methanolic solution of **7** (0.87 g in 30 ml) was heated under reflux with sodium hydroxide (0.4 g) for 30 minutes. After cooling, dilution (water) and filtration, the solid was crystallized from ether to give **21**, yield 0.71 g (83%), mp 148-150°; ^{1}H nmr (deuteriochloroform): 7.80-8.07 (2 H, m), 7.25-7.50 (3 H, m), 6.73 (1 H, d, J = 2 Hz), 6.55 (1 H, d, J = 2 Hz), 4.06 (3 H, s), 3.97 (3 H, s), 3.90 (3 H, s); ir: 3050, 3000, 2965, 2940, 2850, 1610. cm^{-1}; ms: Calcd. for $C_{17}H_{16}N_2O_3$, M^+ = 296. Found FAB: 296.

5-Nitro-2-phenyl-3,6,8-trimethoxyquinoxaline **22**.

The procedure described under **13** was used with **21** (1.5 g). Crystallization from acetone gave **22**, yield 1.3 g (88%), mp 197-200°; ^{1}H nmr (deuteriochloroform/DMSO-d_6): 7.83-8.10 (2 H, m), 7.27-7.53 (3 H, m), 6.65 (1 H, s), 4.06 (6 H, s), 4.03 (3 H, s); ir: 3060, 3010, 2980, 2950, 2850, 1620, 1580 cm^{-1}.

Anal. Calcd. for $C_{17}H_{15}N_3O_5$; C, 59.82; H, 4.43; N, 12.31. Found: C, 59.91; H, 4.41; N, 12.32.

3,6-Dimethoxy-2-phenylquinoxaline-5,8-dione **23**.

The procedure given under **13** was used with **22** (1.7 g). The quinone **23** was crystallized from acetone, yield 1.03 g (70%), mp 223-226°; ^{1}H nmr (deuteriochloroform): 8.03-8.23 (2 H, m), 7.33-7.53 (3 H, m), 6.27 (1 H, s), 4.21 (3 H, s), 3.94 (3 H, s); ir: 3050, 2950, 2840, 1700, 1655 cm^{-1}.

Anal. Calcd. for $C_{16}H_{12}N_2O_4$: C, 64.86; H, 4.08; N, 9.45. Found: C, 64.91; H, 4.07; N, 9.47.

7-Amino-3,6-dimethoxy-2-phenylquinoxaline-5,8-dione **24**.

The method given under **16** (method B) was used with **23** (0.26 g). The product **24** was crystallized from ether, yield 0.08 g (29%), mp 177-179°; ^{1}H nmr (deuteriochloroform/DMSO-d_6): 8.00-8.17 (2 H, m), 7.30-7.47 (3 H, m), 5.03-5.30 (2 H, br), 4.25 (3 H, s), 4.06 (3 H, s); ir: 3460, 3320, 2940, 2840, 1680, 1640, 1600 cm^{-1}.

Anal. Calcd. for $C_{16}H_{13}N_3O_4$: C, 61.72; H, 4.22; N, 13.50. Found: C, 61.82; H, 4.23; N, 13.41.

3-Cyano-6,8-dimethoxy-2-phenylquinoxaline **25**.

A mixture of **8** (2.57 g) and cuprous cyanide (0.85 g, 1.27 equivalents) in DMF (15 ml) was boiled under reflux for 1.25

hours. It was diluted with water, the filtered solid recrystallized from ether to give **25**, yield 1.95 g (90%), mp 227-228°; ^{1}H nmr (deuteriochloroform): 7.80-8.07 (2 H, m), 7.33-7.60 (3 H, m), 7.0 (1 H, d, J = 2 Hz), 6.82 (1 H, d, J = 2 Hz), 4.03 (3 H, s), 3.97 (3 H, s); ir: 3070, 2975, 2945, 2220, 1615 cm^{-1}.

Anal. Calcd. for $C_{17}H_{13}N_3O_2$: C, 70.09; H, 4.50; N, 14.43. Found: C, 70.23; H, 4.69; N, 14.23.

3-Cyano-6,8-dimethoxy-5-nitro-2-phenylquinoxaline **26**.

The procedure given under **13** was used with **25** (0.33 g) and the product crystallized from acetone, yield 0.30 g (79%), mp 262-264°; ^{1}H nmr (deuteriochloroform/DMSO-d_6): 7.90-8.04 (2 H, m), 7.47-7.67 (3 H, m), 7.31 (1 H, s), 4.20 (6 H, s); ir: 3110, 3080, 3060, 2950, 2850, 2240, 1620 cm^{-1}.

Anal. Calcd. for $C_{17}H_{12}N_4O_4$: C, 60.71; H, 3.60; N, 16.66. Found: C, 60.94; H, 3.45; N, 16.78.

3-Cyano-6-methoxy-2-phenylquinoxaline-5,8-dione **27**.

The procedure given under **19** was used with **26** (0.48 g). The product was freed from the nitrophenols by washing with aqueous sodium bicarbonate and crystallized from 9:1 acetone/ligroin to give **27** as light brown crystals, yield 0.12 g (25%), mp 250-253°; ^{1}H nmr (deuteroiochloroform/DMSO-d_6): 7.97-8.20 (2 H, m), 7.47-7.70 (3 H, m), 6.60 (1 H, s), 4.00 (3 H, s); ir: 3060, 2950, 2920, 2850, 2240, 1710, 1655, 1600 cm^{-1}.

Anal. Calcd. for $C_{16}H_9N_3O_3$: C, 65.97; H, 3.11; N, 14.43. Found: C, 66.10; H, 3.21; N, 14.31.

3-Cyano-5,7-dinitro-8-hydroxy-6-methoxy-2-phenylquinoxaline **28**.

The bicarbonate washes from **26** were acidified and the solid chromatographed to give **28** as pale yellow crystals (acetone), yield 0.144 g (24%), mp 141-143°; ^{1}H nmr (deuteriochloroform): 7.97-8.16 (2 H, m), 7.43-7.60 (3 H, m), 5.40-6.0 (1 H, br), 4.10 (3 H, s); ir: 3440, 2960, 1630 cm^{-1}.

The acetate was prepared as given under **18.**

Anal. Calcd. for $C_{18}H_{11}N_5O_7$: C, 52.82; H, 2.71; N, 17.11. Found: 52.89; H, 2.74; N, 17.03.

7-Amino-3-cyano-6-methoxy-2-phenylquinoxaline-5,8-dione **29**.

Method A.

The procedure given under **16** (method B) was used with **28** (0.14 g) and the product crystallized from acetone, yield 0.06 g (41%), mp 264-266°; ^{1}H nmr (deuteriochloroform/DMSO-d_6): 7.70-8.0 (2 H, m), 7.33-7.53 (3 H, m), 6.63 (2 H, br), 3.93 (3 H s); ir: 3480, 3315, 2950, 2850, 2230, 1680 cm^{-1}.

Anal. Calcd. for $C_{16}H_{10}N_4O_3$: C, 62.74; H, 3.30; N, 18.30. Found: C, 62.72; H, 3.33; N, 18.24.

Method B.

A mixture of **27** (0.1 g) and sodium azide (0.1 g) in methanol (5 ml) was heated on a water bath for 5 minutes. It was diluted with water (5 ml), heated with sodium dithionite (0.5 g) for another 5 minutes, cooled and extracted with chloroform. Concentration of the chloroform and crystallization from ether gave the same product **29** described above.

3-Carboxamido-6,8-dimethoxy-2-phenylquinoxaline **30**.

A mixture of **25** (1.0 g) and 1:1 methanol and 10% aqueous sodium hydroxide (30 ml) was boiled under reflux for 2 hours. Cooling, acidification, extraction with chloroform and concentration gave a solid, crystallized from acetone, to give **30** as yellow crystals, yield 0.83 g (78%), mp 273-275°; ^{1}H nmr (deuteriochloroform/DMSO-d_6): 7.60-7.90 (2 H, br), 7.60-7.78 (2 H, m), 7.27-7.53 (3 H, m), 7.00 (1 H, d, J = 2 Hz), 6.82 (1 H, d, J = 2 Hz), 4.00 (3 H, s), 3.93 (3 H, s); ir: 3480, 3360, 3060, 2960, 2840, 1695 cm^{-1}.

Anal. Calcd. for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.83; H, 4.79; N, 13.47. A more satisfactory value for hydrogen could not be obtained.

3-Carbomethoxy-6,8-dimethoxy-2-phenylquinoxaline **31**.

A 1:1 methanol/sulfuric acid mixture (30 ml) was added to **30** (2.0 g), and heated at 90° for 1 hour. Adding ice, filtering the solid and crystallization from ether gave yellow crystals of **31**, yield 1.30 g (62%), mp 212-214°; ^{1}H nmr (deuteriochloroform): 7.60-7.70 (2 H, m), 7.33-7.54 (3 H, m), 7.07 (1 H, d, J = 2 Hz), 6.80 (1 H, d, J = 2 Hz), 4.03 (3 H, s), 3.95 (3 H, s), 3.83 (3 H, s); ir: 3000, 2970, 2940, 2830, 1740, 1615 cm^{-1}.

Anal. Calcd. for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64. Found: C, 66.63; H, 5.01; N, 8.66.

3-Carbomethoxy-6,8-dimethoxy-5-nitro-2-phenylquinoxaline **32**.

The procedure given under **13** was used with **31** (1.0 g) and crystallization (acetone) gave **32**, yield 0.63 g (55%); mp 256-257°; ^{1}H nmr (deuteriochloroform/DMSO-d_6): 7.35-7.70 (5 H, m), 7.25 (1 H, s), 4.13 (6 H, s), 3.76 (3 H, s); ir: 2950, 2880, 2840, 1730, 1615 cm^{-1}.

Anal. Calcd. for $C_{18}H_{15}N_3O_6$: C, 58.53; H, 4.09; N, 11.38. Found: C, 58.61; H, 4.09; N, 11.40.

3-Carbomethoxy-6-methoxy-2-phenylquinoxaline-5,8-dione **33**.

The procedure given under **15** was used with **32** (0.48 g) and the product **33** crystallized from ether, yield 0.29 g (70%), mp 209-211°; ^{1}H nmr (deuteriochloroform): 7.53-7.77 (2 H, m), 7.23-7.53 (3 H, m), 6.40 (1 H, s), 3.93 (3 H, s), 3.83 (3 H, s); ir: 2950, 1735, 1700, 1655 cm^{-1}.

Anal. Calcd. for $C_{17}H_{12}N_2O_5$: C, 62.96; H, 3.73; N, 8.64. Found: C, 62.84; H, 3.75; N, 8.59.

7-Amino-3-carbomethoxy-6-methoxy-2-phenylquinoxaline-5,8-dione **34**.

The procedure given under **16** (method b) was used with **33** (0.1 g) and the product **34** crystallized from acetone, yield 0.037 g (36%), mp 197-203° dec; ^{1}H nmr (deuteriochloroform/DMSO-d_6): 7.37-7.70 (5 H, m), 6.65 (2 H, br), 3.94 (3 H, s), 3.79 (3 H, s); ir: 3430, 3260, 3280, 2950, 1730, 1685, 1635 cm^{-1}.

Anal. Calcd. for $C_{17}H_{13}N_3O_5$: C, 60.17; H, 3.86; N, 12.39. Found: C, 59.98; H, 3.90; N, 12.08.

7-Bromo-3-chloro-6-methoxy-2-phenylquinoxaline-5,8-dione **35**.

Bromine (0.2 ml) was added drop-wise with stirring to pyridine (3 ml) at 0°. This reagent was added to **20** (0.84 g) in pyridine (5 ml) at 0-5° with stirring. After 45 minutes, 2*N* hydrochloric acid (50 ml) was added and the mixture extracted with chloroform and the extract, after washing with sodium bisulfite, followed by sodium bicarbonate was concentrated to dryness and the solid crystallized from ether/ligroin gave **35**, yield 0.58 g (55%), mp 240-250° dec; ^{1}H nmr (deuteriochloroform): 7.88-7.97 (2 H, m), 7.47-7.55 (3 H, m), 4.37 (3 H, s), ms: Calcd. for $C_{15}H_8N_2O_3ClBr$: M^+ 379. Found: 379.

7-Azido-8-methoxy-4-phenyl-1,5-tetrazoloquinoxaline-6,9-dione **36**.

A solution of **35** (0.44 g) in dimethylformamide (10 ml), and sodium azide (0.1 g) was stirred for 5 minutes. It was diluted with water, the solid filtered and crystallized from ether to yield 0.37 g (92%) of **36** as dark-brown crystals; ^{1}H nmr (deuteriochloroform): 8.60-8.80 (2 H, m) 7.43-7.67 (3 H, m), 4.20 (3 H, s); ir: 3400, 2940, 2860, 2120, 1680, 1675 cm^{-1}.

Compound **36** was used in the next reaction without further purification.

7-Amino-8-methoxy-4-phenyl-1,5-tetrazoloquinoxaline-6,9-dione **37**.

The procedure given under **16** (method a) was used with **36** (0.2 g). The product, **37** was crystallized from acetone/ligroin (3:1), yield 0.062 g (90%), mp 190-192° dec; ^{1}H nmr (deuteriochloroform) 8.53-8.80 (2 H, m), 7.40-7.67 (3 H, m), 6.75-7.03 (2 H, br), 3.83 (3 H, s); ir: 3455, 3340, 2940, 1710, 1645 cm^{-1}.

Anal. Calcd. for $C_{15}H_{10}N_6O_3$: C, 55.90; H, 3.13; N, 26.08. Found: C, 56.20; H, 3.28; N, 25.98.

5,7-Dimethoxy-8-nitro-3-phenylquinoxalin-2(1*H*)-one **38**.

The procedure used under **13** was used with **5** (0.5 g). Crude **38** was partitioned between chloroform and aqueous ammonia, the aqueous layer was acidified, filtered, and the solid crystallized from acetone, yield 0.51 g (90%), mp 300° dec; ^{1}H nmr (DMSO-d_6): 8.13-8.36 (2 H, m), 7.43-7.63 (3 H, m), 6.90 (1 H, s), 4.20 (3 H, s), 4.17 (3 H, s); ir: 3120, 2980, 2930, 1660 cm^{-1}; ms: Calcd, 327. Found: FAB $[M+1]^+ = 328$.

Anal. Calcd. for $C_{16}H_{13}N_3O_5$: C, 58.71; H, 4.00; N, 12.84. Found: C, 58.52; H, 4.12; N, 12.66.

8-Amino-5,7-dimethoxy-3-phenylquinoxalin-2(1*H*)-one **38a**.

A mixture of **38** (1.57 g), methanol (100 ml), 5% sodium bicarbonate (30 ml) and sodium dithionite (4.0 g), was refluxed for 30 minutes. It was diluted with water, the solid filtered and crystallized from acetone to give **38a** as orange needles, yield 0.92 g (64%), mp 245-250° dec; ^{1}H nmr (deuteriochloroform-/DMSO-d_6): 8.07-8.24 (2 H, m), 7.24-7.39 (m), 6.42 (1 H, s), 3.90 (6 H, s); ir: 3450, 3385, 3120, 3000, 2935, 2840, 1660 cm^{-1}; ms: Calcd. for $C_{16}H_{15}N_3O_3$: 297. Found: FAB $M^+ = 297$.

7-Methoxy-3-phenyl-2(1*H*)-quinoxalinone-5,8-dione **39**.

To **38** (0.3 g) in methanol (15 ml) was added 1*M* sulfuric acid (20 ml) containing sodium periodate (0.3 g). After stirring for 2-3 minutes, the reddish-brown solid that separated was filtered and crystallized from acetone to give **39**, yield 0.25 g (87%), mp 268-270° dec; ^{1}H nmr (deuteriochloroform/DMSO-d_6): 8.33-8.47 (2 H, m), 7.37-7.57 (3 H, m), 6.21 (1 H, s), 3.90 (3 H, s); ir: 3060, 2920, 2850, 1685, 1655, 1640 cm^{-1}; ms: Calcd. for $C_{15}H_{10}N_2O_4$: 282. Found: EI 282.

Anal. Calcd. for $C_{15}H_{10}N_2O_4$: C, 63.83; H, 3.57; N, 9.93. Found: C, 63.71; H, 3.31; N, 9.72.

6-Bromo-7-methoxy-3-phenyl-2(1*H*)-quinoxalinone-5,8-dione **40**.

Conditions described under **35** were used with **39** (0.6 g) and the product **40** was crystallized from 9:1 ether/acetone, yield 0.64 g (84%), mp 245-250° dec; ^{1}H nmr (deuteriochloroform): 8.20-8.45 (2 H, m), 7.27-7.53 (3 H, m), 4.18 (3 H, s); ir: 3050, 2960, 1680, 1660, 1650 cm^{-1}. Calcd. for $C_{15}H_9N_2O_4Br$: 361. Found: EI 361.

6-Amino-7-methoxy-3-phenyl-2(1*H*)-quinoxalinone-5,8-dione **41**.

The procedure given under **29** (method B) was used with **40** (0.2 g) and crystallization from acetone gave dark gray crystals of **41**, yield 0.087 g (52%), mp 270°; ^{1}H nmr (deuterichloroform/DMSO-d_6): 8.10-8.37 (2 H, m), 7.17-7.50 (3 H, m), 6.97 (2 H, br), 3.76 (3 H, s); ir: 3420, 3280, 3260, 3180, 3150, 3000, 2940, 1695, 1655, 1640 cm^{-1}.

Anal. Calcd. for $C_{15}H_{11}N_3O_4$: C, 60.60; H, 3.73; N, 14.14. Found: C, 60.50; H, 3.89; N, 13.95.

7-Methoxy-1-methyl-3-phenyl-2(1*H*)-quinoxalinone-5,8-dione **42**.

To **39** (0.75 g) in chloroform (25 ml) was added diazomethane in ether (3 ml). After 2 minutes, the solution was concentrated to dryness and the product crystallized from ether to give **42** as orange crystals, yield 0.71 g (90%), mp 250-255° dec; ^{1}H nmr (deuteriochloroform): 8.33-8.50 (2 H, m), 7.30-7.57 (3 H, m), 6.10 (1 H, s), 4.00 (3 H, s), 3.90 (3 H, s); ir: 3070, 2950, 1690, 1670, 1650 cm^{-1}; ms: Calcd. for $C_{16}H_{12}N_2O_4$: 296. Found: EI 296 (100).

Anal. Calcd. for $C_{16}H_{12}N_2O_4$: C, 64.86; H, 4.08; N, 9.46. Found: C, 64.59; H, 4.11; N, 9.33.

6-Bromo-7-methoxy-1-methyl-3-phenyl-2(1*H*)-quinoxalinone-5,8-dione **43**.

The details given under **35** were used with **42** (0.6 g) and crystallization from ether gave **43** as red crystals, yield 0.64 g (75%), mp 202-204°; ^{1}H nmr (deuteriochloroform): 8.30-8.50 (2 H, m), 7.33-7.53 (3 H, m), 4.15 (3 H, s), 3.90 (3 H, s); ir: 3120, 3070, 2970, 1680, 1670, 1660 cm^{-1}; ms: Calcd. for $C_{16}H_{11}N_2O_4Br$: 375. Found: EI 375.

6-Amino-7-methoxy-1-methyl-3-phenyl-2(1*H*)-quinoxalinone-5,8-dione **44**.

The procedure given under **29** (method B) was used with **43** (0.2 g) and the product crystallized from acetone to give purple crystals of **44**, yield 0.078 g (47%), mp 217-218°; ^{1}H nmr (deuteriochloroform/DMSO-d_6): 8.21-8.47 (2 H, m), 7.21-7.53 (3 H, m), 5.03-5.36 (2 H, br), 4.00 (3 H, s), 3.90 (3 H, s); ir: 3460, 3350, 2960, 2930, 1675, 1650 cm^{-1}.

Anal. Calcd. for $C_{16}H_{13}N_3O_4$: C, 61.73; H, 4.21; N, 13.50. Found: C, 61.68; H, 4.30; N, 13.25.

6,8-Dimethoxy-2-phenyl-3-*N*-piperidinylquinoxaline **45**.

A mixture of **7** (0.164 g) and piperidine (0.2 ml) in DMF (6 ml) was heated under reflux for 1.5 hours. Water was added, and the solid filtered and crystallized from ether to give pale-yellow crystals of **45**, yield 0.154 g (81%), mp 145-146°; ^{1}H nmr (deuteriochloroform): 7.77-8.00 (2 H, m), 7.27-7.53 (3 H, m), 6.73 (1 H, d, J = 3 Hz), 6.47 (1 H, d, J = 3 Hz), 3.95 (3 H, s), 3.90 (3 H, s), 3.87 (4 H, br), 1.54 (6 H, br); ir: 3450, 2935, 2840, 2820, 1610 cm^{-1}.

Anal. Calcd. for $C_{21}H_{23}N_3O_2$: C, 72.18; H, 6.63; N, 12.02. Found: C, 72.28; H, 6.67; N, 12.09.

6-Methoxy-2-phenyl-3-*N*-piperidinylquinoxaline-5,8-dione **46**.

Piperidine (0.1 ml, 1.1 equivalents) was added drop-wise with stirring, to **19** (0.3 g) in dimethylformamide (6 ml) at 0°. The red solution was diluted with water (25 ml), the solid filtered, and crystallized from 2:1 acetone/ligroin, yield 0.287 g (83%), mp 235-239°; ^{1}H nmr (deuteriochloroform): 7.70-7.93 (2 H, m), 7.33-7.53 (3 H, m), 6.20 (1 H, s), 3.90 (3 H, s), 3.37 (4 H, br), 1.60 (6 H, br); ir: 3050, 3010, 2940, 2840, 1690, 1645 cm^{-1}.

Anal. Calcd. for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03. Found: C, 68.80; H, 5.37; N, 11.99.

7-Bromo-3-chloro-2-phenyl-6-*N*-piperidinylquinoxaline-5,8-dione **47**.

The procedure given under **46** was used with **35** (0.1 g) and piperidine (0.03 ml, 1.2 equivalents). Product **47** was crystallized from 2:1 acetone/ligroin, yield 0.075 g (60%), mp 171-172° dec; ^{1}H nmr (deuteriochloroform): 7.73-7.97 (2 H, m), 7.33-7.50 (3 H, m), 3.57 (4 H, br), 1.73 (6 H, br); ir: 3050, 2930, 2830, 1685, 1645 cm^{-1}.

Anal. Calcd. for $C_{19}H_{15}N_3O_2BrCl$: C, 52.84; H, 3.50; N, 9.73. Found: C, 52.91; H, 3.46; N, 9.66.

3,6-Di-*N*-piperidinyl-2-phenylquinoxaline-5,8-dione **48**.

The reaction described under **46** was repeated with **19** (0.24 g) and piperidine (0.2 ml). The yellow solution changed to red and then to dark green. The product, after workup was crystallized from 2:1 acetone/ligroin, yield 0.23 g (71%), mp 223-225°; ^{1}H nmr (deuteriochloroform): 7.70-7.93 (2 H, m), 7.23-7.50 (3 H, m), 6.03 (1 H, s), 3.23-3.60 (8 H, br), 1.30-1.73 (12 H, br); ir: 3040, 2935, 2840, 1680, 1630 cm^{-1}.

Anal. Calcd. for $C_{24}H_{26}N_4O_2$: C, 71.62; H, 6.51; N, 13.92. Found: C, 71.84; H, 6.35; N, 13.75.

7-Bromo-3,6-di-*N*-piperidinyl-2-phenylquinoxaline-5,8-dione **49**.

The reaction described under **46** was repeated with **35** (0.214 g) and piperidine (0.12 ml, 3 equivalents). Product **49** was crystallized from acetone/ligroin, yield: 0.176 g (65%), mp 168-174° dec; ^{1}H nmr (deuteriochloroform): 7.63-7.87 (2 H, m), 7.27-7.50 (3 H, m), 3.20-3.60 (8 H, br), 1.33-1.90 (12 H, br); ir: 3050, 2930, 2845, 1680, 1640 cm^{-1}.

Anal. Calcd. for $C_{24}H_{25}N_4O_2Br$: C, 59.98; H, 5.24; N, 11.66. Found: C, 59.77; H, 5.29; N, 11.55.

7-Bromo-3-cyano-6-methoxy-2-phenylquinoxaline-5,8-dione **50**.

The procedure given under **35** was used with **27** (0.11 g), with crystallization from ether, yield 0.065 g (47%), mp 195-198°; ^{1}H nmr (deuteriochloroform): 7.85-8.00 (2 H, m), 7.47-7.60 (3 H, m), 4.37 (3 H, s); ms: Calcd. for $C_{16}H_8N_3O_3Br$: M^+ 371. Found: 371.

Anal. Calcd. for $C_{16}H_8N_3O_3Br$: C, 51.77; H, 2.17; N, 11.31. Found: C, 51.51; H, 2.21; N, 11.01

6-Amino-7-bromo-3-chloro-2-phenylquinoxaline-5,8-dione **51**.

A solution of **23** (0.13 g) in chloroform was stirred with ammonia in chloroform (obtained by shaking 30% aqueous ammonia with chloroform). After 20 minutes the mixture was diluted with water, made acidic and the solvent layer concentrated to dryness. Crystallization from benzene gave **57** as red crystals, yield 0.1 g (73%), mp 272-276° dec; ^{1}H nmr (deterichloroform): 7.83-7.90 (2 H, m), 7.33-7.60 (3 H, m), 4.83 (2 H, s); ir: 3460, 3325, 2920, 2850, 1695, 1655, 1605, 1530, 1510, 1370, 1300, 1200, 1160, 920, 730, 690 cm^{-1}.

Anal. Calcd. for $C_{14}H_7N_3O_2BrCl$: C, 46.12; H, 1.94; N, 11.53. Found: C, 46.05; H, 2.02; N, 11.39.

6-Amino-7-bromo-3-cyano-2-phenylquinoxaline-5,8-dione **52**.

The reaction described under **51** was repeated with **50** (0.1 g) and the product crystallized from benzene to give **52** as red crystals, yield 0.059 g (62%), mp 276-279° dec; ^{1}H nmr (deuteriochloroform): 7.93-8.10 (2 H, m), 7.33-7.60 (3 H, m); ir: 3440, 3340, 2240, 1695, 1655 cm^{-1}.

Anal. Calcd. for $C_{15}H_7N_4O_2Br$: C, 50.84; H, 1.99; N, 15.81. Found: C, 50.64; H, 1.92; N, 15.63.

Tests For Biological Activity.

L-1210 Cytotoxicity Test.

The method of Thayer *et al.*, was used [30]. Two ml of the cell suspension (150,000 cells per ml), maintained at 37° under carbon dioxide atmosphere, was placed in each well of a Becton-Dickinson deep-well plate (24 wells/plate). Solutions of the compounds in DMSO (2 mg/ml) were dispensed (0.01 ml) to give five levels covering the activity range, each differing by two-fold and each in quadruplicate, into the wells containing the L1210 cells. After 48 hours at 37°, 1 ml of the culture was mixed with 1 ml of trypan blue, 0.1 ml of this was placed on a hemocytometer (Fisher), and the unstained, viable cells present in the five gridded areas were counted. This number x 4000 gives the number of cells in 1 ml of the culture in the well.

Controls with and without DMSO, and an active positive control which, in this case, was taxol, were also run. The IC_{50} was determined from the plot of log [concentration] *vs.* % inhibition and the latter was obtained by the equation:

$$\%\text{inhibition} = 1 - (T_d - T_o / T_c - T_o),$$

where T_d is the # of cells per ml treated samples, T_o is the initial # of cells, and T_c is the average # of cells per ml of the controls.

Antibiotic Assay.

The disk-plate method of antibiotic assay is used. The plates were prepared using Difco Streptomycin assay agar (10 ml per plate), inoculated with spores of *Bacillus subtilis* (Fisher) and stored inverted at 5° until use.

Aliquots (50 m) of solutions of the compounds in DMSO containing 100, 20, 4, 0.8, and 0.016 m, were pipetted onto the assay discs (Schleicher and Schuell, 12.5 mm) and the discs placed, equally spaced, on the agar plates (5 levels per plate and in quadruplicate). After 8 hours at 5° for diffusion, the plates were kept at 37°. After 20 hours, the diameters of the zones of inhibition were measured using a ruler. A plot of log [concentration] *vs.* zone diameter (mm), which was subjected to linear regression, was generated. The MIC (minimum inhibitory concentration) is that concentration giving a barely perceptible zone (13 mm). Standard error for the slope was calculated as per Strike [31].

Inhibition of Root Growth.

The method of Noel *et al.* was used [32]. Seeds of *Lepidium sativum* (2.5 g) (Charles A. Hart Co.) soaked in water (15 ml) for 15-20 minutes were spread across phage-typing petri dishes (Falcon) containing 20 ml of 0.6% agar (4-5 seeds in each square). The plates were incubated at 27° for 24 hours for the seeds to develop a 2-3 cm radical.

Solutions of the compounds in DMSO were made, so that when 0.2 ml was added to 20 ml of agar gave final concentrations of 20, 10, 4, 2, and 0.8 µg/ml. The agar with the compound and the control agar were poured into the plates and cooled.

The rooted seedlings were placed on the plates in two rows of four, with the tip of each radical at the crossing of two grid lines. The plates in bundles of 10 were kept, stacked upright to allow geotropic root growth for 24 hours at 27°, and the new root growth for each seedling was measured with a ruler starting from the cross point. The averaged values were plotted as log [concentration] *vs.* % inhibition, and a regression line was generated from which the IC_{50} values were obtained.

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* To whom enquiries should be addressed

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