mp 173–174 °C); ^{1}H NMR ($CDCl_3$, free base) δ 7.25 (m, 5, Ar H), 6.78 (m, 3, Ar H), 3.87 (m, 1, CH), 3.84, 3.83 (2 s, 6, CH_3O), 3.30 (m, 2, CH_2), 2.04 (br s, 2, NH_2). Anal. ($C_{16}H_{20}NClO_2$) C, H, N.

2-(3,4-Dihydroxyphenyl)-2-phenylethylamine Hydrobromide (4). A solution of compound **13**·HCl (0.08 g, 0.27 mmol) in 1 mL of 48% HBr was stirred for 1.5 h at reflux under a nitrogen atmosphere. After removal of the HBr by rotary evaporation, the residue was recrystallized from 2-propanol/ethyl acetate: yield 55 mg (65%); mp 232–235 °C; ^{1}H NMR (D_2O) δ 6.69 (s, 5, Ar H), 6.33 (m, 3, Ar H), 4.47 (dd, 1, CH), 4.05 (d, 2, CH_2). Anal. ($C_{14}H_{16}BrNO_2$) C, H, N.

X-ray Analysis. The X-ray analysis of a single crystal of the pure (α*S*,2*R*)-**10a**·CH_3SO_3H diastereomer was performed.

Crystal data: $C_{19}H_{25}NO_2{\cdot}CH_3SO_3H$, MW = 395.65, orthorhombic, a = 12.73 (1) Å, b = 9.134 (4) Å, c = 17.881 (8) Å, V = 2080 (2) Å^3, Z = 4, ρ_{calcd} = 1.27 g/cm^3, $F(000)$ = 848, μ(Cu Kα) = 15.28, space group $P2_12_12_1$ from systematic absences.

Data Collection. Crystallographic data were collected with Cu Ka X-rays and a monochromator on a Nicolet P3 four-circle diffractometer, with the θ–2θ scan technique out to a 2θ of 116.0°. A variable scan rate was used with a maximum of 29.30°/min and a minimum of 7.23°/min. The scan range was from 1.2° < $K\alpha_1$ to 1.2° > $K\alpha_2$; the time that backgrounds at both ends of the scan range was counted equivalent to the scan time. Three standard reflections were measured every 50 reflections.

Structure Analysis. Twenty-two reflections were rejected as systematically absent of the 1649 reflections collected, leaving 1627 unique reflections, of which 1326 met the condition of $F_o > 5\sigma(F_o)$ and were considered observed. The structure was solved by using the MULTAN80 program and refined by SHELX76 to a final R of 0.0789 with the hydrogens fixed in their calculated positions. A final difference map showed no peaks greater than 0.40 e/Å^3. Absolute configuration is (*S*)-(−)-*N*-(α-methylbenzyl)-2(*R*)-(3,4-dimethoxyphenyl)propylamine (Figure 1.).

Pharmacology. The procedure for the dopamine-sensitive rat retinal adenylate cyclase assay was performed as follows: Rat retinas were homogenized in 150 vol/wt of 2.0 mM Tris·HCl, pH 7.4, with 2 mM EDTA with a Teflon–glass homogenizer. Each reaction mixture contained the following final concentrations in a volume of 0.2 mL: 2 mM $MgSO_4{\cdot}7\ H_2O$, 0.5 mM EGTA, 1 mM IBMX, 0.01 mM GTP, 80 mM Tris·HCl (pH 7.4), 0.5 mM ATP with approximately 5 × 10^6 DPM [^{32}P]ATP and 20–30 μg of retinal homogenate protein. Following an incubation of 20 min at 30 °C, the reaction was terminated by adding 200 μL of a solution containing 1% SDS, 20 mM ATP, 0.7 mM cyclic AMP with 1.0 × 10^4 DPM [^{3}H]cyclic AMP in 80 mM Tris·HCl, pH 7.4, and heating to 85 °C for 2 min. Cyclic AMP was isolated from the mixture by using the column chromatographic technique of Salomon.[19]

Acknowledgment. This work was supported by NIH Grants HL-31106 and MH-42705.

Supplementary Material Available: The atom locations and temperature factors for the X-ray determination of **10a** (2 pages) are available. Ordering information is given on any current masthead page.

(17) Maryanoff, B.; Nortey, S.; Gardocki, J. *J. Med. Chem.* **1984**, *27*, 1067.
(18) Deshpande, V.; Nargund, K. *Chem. Abstr.* **1958**, *52*, 7183*f*.
(19) Salomon, *Adv. Cyclic Nucleotide Res.* **1979**, *10*, 35.

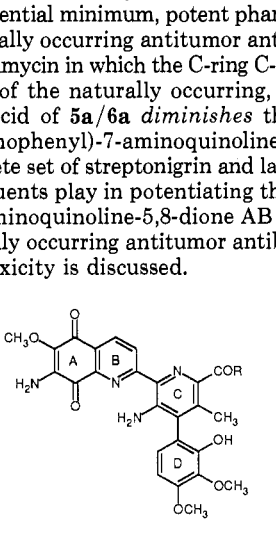
Streptonigrin and Lavendamycin Partial Structures. Probes for the Minimum, Potent Pharmacophore of Streptonigrin, Lavendamycin, and Synthetic Quinoline-5,8-diones

Dale L. Boger,*†‖ Masami Yasuda,† Lester A. Mitscher,‡ Steven D. Drake,‡ Paul A. Kitos,§ and Sandra Collins Thompson§

Departments of Chemistry and Medicinal Chemistry, Purdue University, West Lafayette, Indiana 47907, Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045–2500, and Department of Biochemistry, University of Kansas, Lawrence, Kansas 66045–2500. *Received April 2, 1987*

The preparation and evaluation of 7-amino-5,8-dioxo-2-(2′-pyridyl)quinoline-6′-carboxylic acid (**5a**) and 7-amino-2-(2′-aminophenyl)-5,8-dioxoquinoline-5′-carboxylic acid (**6a**) constituting potential minimum, potent pharmacophores of streptonigrin (**1a**) and lavendamycin (**2a**), two structurally related naturally occurring antitumor antibiotics, are detailed. In contrast to observations associated with streptonigrin and lavendamycin in which the C-ring C-6′ carboxylic acid *potentiates* the antitumor, antimicrobial, and cytotoxic properties of the naturally occurring, substituted 7-aminoquinoline-5,8-dione AB ring systems, the C-6′/C-5′ carboxylic acid of **5a**/**6a** *diminishes* the observed antimicrobial and cytotoxic properties of the 2-(2′-pyridyl)- and 2-(2′-aminophenyl)-7-aminoquinoline-5,8-diones. A direct comparison of the antimicrobial and cytotoxic properties of a complete set of streptonigrin and lavendamycin partial structures is detailed in efforts to define the role peripheral substituents play in potentiating the biological properties of the naturally occurring and synthetic agents bearing the 7-aminoquinoline-5,8-dione AB ring system and in efforts to define the minimum, potent pharmacophore of the naturally occurring antitumor antibiotics. The relationship of these observations to a chemical mechanism of cellular toxicity is discussed.

Streptonigrin (**1a**), a highly substituted and highly functionalized 7-aminoquinoline-5,8-dione first isolated from *Streptomyces flocculus*,[2-7] has been shown to possess potent cytotoxic properties, confirmed broad spectrum antitumor activity, and in vitro and in vivo antiviral properties[19] and to display potent, broad spectrum antimicrobial properties.[8-20] Early detailed investigations[14-18] defined the broad spectrum antitumor activity of streptonigrin against sarcoma 180, mammary adenosarcoma 755, Lewis lung carcinoma, Ridgway and Wagner osteogenic carcinoma, Harding–Passey melanoma, Walker 256 car-



1a, R = OH Streptonigrin
1b, R = OCH_3 Streptonigrin methyl ester
1c, R = $NHNH_2$ Streptonigrin hydrazide

† Purdue University.
‡ Department of Medicinal Chemistry, University of Kansas.
§ Department of Biochemistry, University of Kansas.

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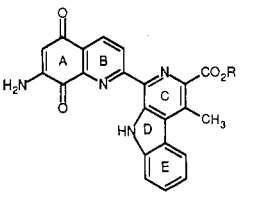
cinosarcoma, mouse mammary carcinoma, Flexner–Jabling carcinoma, Iglesias ovarian tumor, and human herpes simplex 1 grown in rats and complemented the early investigations detailing the broad spectrum antimicrobial properties of streptonigrin against Gram-positive, Gram-negative, acid-fast bacteria, and fungal organisms. Its potential clinical use has been precluded only by its equally potent toxicity.[14,18] Continued and recent efforts have explored the clinical use of streptonigrin in combination chemotherapy with additional agents including vincristine and bleomycin.[21-24]

The antimicrobial and antitumor properties of lavendamycin (**2a**), a 7-aminoquinoline-5,8-dione isolated from *Streptomyces lavendulae*[25] and shown to be structurally[25,26] and biosynthetically[27] related to streptonigrin, have been examined. With notable exceptions lavendamycin was found to be comparable to albeit less potent than streptonigrin in its observed spectrum of activity.[25]



2a, R = H Lavendamycin
2b, R = CH_3 Lavendamycin methyl ester

Present efforts have shown that streptonigrin cellular toxicity may be attributed to the depletion of NADPH/NADH,[8-11] the uncoupling of oxidative phosphorylation, and/or the single-strand cleavage of DNA.[8-11,28,29] This latter effect, which disrupts DNA synthesis and which has been studied in cell-free systems, has been actively pursued as the potential mechanism of streptonigrin antitumor activity.[8-11,28-42]

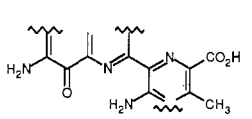
(1) National Institutes of Health research career development award recipient, 1983–1988 (CA 01134). Alfred P. Sloan research fellow recipient, 1985–1989.

(2) Streptonigrin isolation: Rao, K. V.; Cullen, W. P. *Antibiot. Annu.* **1959**, 950. Identical with bruneomycin (*Actinomyces albus var. bruneomycini*): Kudrina, E. S.; Olkhovatova, O. L.; Murav'eva, L. I.; Gauze, G. F. *Antibiotiki (Moscow)* **1966**, *11*, 400. Brazhnikova, M. G.; Ponomarenko, V. I.; Kovsharova, I. N.; Kruglyak, E. B.; Proshlyakova, V. V. *Antibiotiki (Moscow)* **1968**, *13*, 99. Identical with rufochromomycin (*Streptomyces rufochromogenus*): *Chem. Abstr.* **1961**, *55*, 25158.
(3) Streptonigrin structure identification: Rao, K. V.; Biemann, K.; Woodward, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2532.
(4) Streptonigrin single-crystal X-ray structure determination: Chiu, Y.-Y. H.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1975**, *97*, 2525.
(5) Streptonigrin atropisomers: Dholakia, S.; Gillard, R. D. *Tetrahedron* **1981**, *37*, 2929.
(6) Streptonigrin total syntheses: (a) Weinreb, S. M.; Basha, F. Z.; Hibino, S.; Khatri, N. A.; Kim, D.; Pye, W. E.; Wu, T.-T. *J. Am. Chem. Soc.* **1982**, *104*, 536. (b) Kende, A. S.; Lorah, D. P.; Boatman, R. J. *J. Am. Chem. Soc.* **1981**, *103*, 1271. (c) Boger, D. L.; Panek, J. S. *J. Am. Chem. Soc.* **1985**, *107*, 5746; *J. Org. Chem.* **1983**, *48*, 621.
(7) Gould, S. J.; Weinreb, S. M. *Fortschr. Chem. Org. Naturst.* **1982**, *41*, 77.
(8) Lown, J. W. *Mol. Cell. Biochem.* **1983**, *55*, 17.
(9) Lown, J. W. In *Bioorganic Chemistry*; Van Tamelen, E. E., Ed.; Academic: New York, 1977; pp 95–121.
(10) Hajdu, J. *Metal Ions in Biological Systems*; Marcel Dekker: New York, 1985; Vol. 19, Chapter 3.
(11) Bhuyan, B. K. In *Antibiotics I. Mechanism of Action*; Gottlieb, I. D., Shaw, P. D., Eds.; Springer-Verlag: New York, 1967; pp 173–180.
(12) Rao, K. V. *Cancer Chemother. Rep., Part 2* **1974**, *4*(3), 11.
(13) Driscoll, J. S.; Hazard, G. F., Jr.; Wood, H. B., Jr.; Boldin, A. *Cancer Chemother. Rep., Part 2* **1974**, *4*(2), 1.
(14) Wilson, W. L.; Labra, C.; Barrist, E. *Antibiot. Chemother. (Washington, D.C.)* **1961**, *11*, 147.
(15) Oleson, J. J.; Calderella, L. A.; Mjos, K. J.; Reith, A. R.; Thie, R. S.; Toplin, I. *Antibiot. Chemother. (Washington, D.C.)* **1961**, *11*, 158.
(16) Teller, M. N.; Wagshul, S. F.; Woolley, G. W. *Antibiot. Chemother. (Washington, D.C.)* **1961**, *11*, 165.
(17) Reilly, H. C.; Sugiura, K. *Antibiot. Chemother. (Washington, D.C.)* **1961**, *11*, 174.
(18) Hackethal, C. A.; Golbey, R. B.; Tan, T. C.; Karnofsky, D. A.; Burchenal, J. H. *Antibiot. Chemother. (Washington, D.C.)* **1961**, *11*, 178.
(19) Chirigos, M. A.; Pearson, J. W.; Papas, T. S.; Woods, W. A.; Wood, H. B., Jr.; Spahn, G. *Cancer Chemother. Rep., Part 1* **1973**, *57*, 305.
(20) McBride, T. J.; Oleson, J. J.; Woolf, D. *Cancer Res.* **1966**, *26A*, 727.
(21) Kuang, D. T.; Whittington, R. M.; Spenser, H. H.; Patno, M. E. *Cancer (Philadelphia)* **1969**, *23*, 597.
(22) Nissen, N. I.; Pajak, T.; Glidewell, O.; Blom, H.; Flaherty, M.; Hayes, D.; McIntyre, R.; Holland, J. F. *Cancer Treat. Rep.* **1977**, *61*, 1097. Forcier, R. J.; McIntyre, O. R.; Nissen, N. I.; Pajak, T. F.; Glidewell, O.; Holland, J. F. *Med. Pediatr. Oncol.* **1978**, *4*, 351.
(23) Banzet, P.; Jacquillat, C.; Civatte, J.; Puissant, A.; Maral, J.; Chanstang, C.; Israel, L.; Belaich, S.; Jourdain, J. C.; Weil, M.; Auclerc, G. *Cancer (Philadelphia)* **1978**, *41*, 1240.
(24) Gout-Lemerle, M.; Rodary, C.; Sarrazin, D. *Arch. Fr. Pediatr.* **1976**, *33*, 527.
(25) Lavendamycin isolation and antimicrobial and antitumor testing: Balitz, D. M.; Bush, J. A.; Bradner, W. T.; Doyle, T. W.; O'Herron, F. A.; Nettleton, D. E. *J. Antibiot.* **1982**, *35*, 259.
(26) Lavendamycin structure determination: Doyle, T. W.; Balitz, D. M.; Grulich, R. E.; Nettleton, D. E.; Gould, S. J.; Tann, C.-h.; Moews, A. E. *Tetrahedron Lett.* **1981**, *22*, 4595.
(27) Lavendamycin biosynthesis: Erickson, W. R.; Gould, S. J. *J. Am. Chem. Soc.* **1985**, *107*, 5831. Erickson, W. R.; Gould, S. J. *J. Am. Chem. Soc.* **1987**, *109*, 620.
(28) Lavendamycin total syntheses: (a) Kende, A. S.; Ebetino, F. H. *Tetrahedron Lett.* **1984**, *25*, 923. (b) Hibino, S.; Okazaki, M.; Ichikawa, M.; Sato, K.; Ishizu, T. *Heterocycles* **1985**, *23*, 261. (c) Boger, D. L.; Duff, S. R.; Panek, J. S.; Yasuda, M. *J. Org. Chem.* **1985**, *50*, 5790. (d) Rao, A. V. R.; Chavan, S. P.; Sivadasan, L. *Tetrahedron* **1986**, *42*, 5065.
(29) Bachur, N. R.; Gordon, S. L.; Gee, M. V. *Cancer Res.* **1978**, *38*, 1745. Bachur, N. R.; Gordon, S. L.; Gee, M. V.; Kon, H. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 954.
(30) Cone, R.; Hasan, S. K.; Lown, J. W.; Morgan, A. R. *Can. J. Biochem.* **1976**, *54*, 219. No evidence was found for streptonigrin–DNA interaction in the presence or absence of Cu(II).
(31) Lown, J. W.; Sim, S.-K. *Can. J. Biochem.* **1976**, *54*, 446.
(32) Lown, J. W.; Sim, S.-K.; Chen, H.-H. *Can. J. Biochem.* **1978**, *56*, 1042.
(33) Lown, J. W.; Sim, S.-K. *Can. J. Chem.* **1976**, *54*, 2563.
(34) Divalent metal cations including Mn(II), Zn(II), Cd(II), and Cu(II) promote the association of streptonigrin with calf thymus DNA and enhance its antimicrobial activity against *Escherichia coli*; cf.: White, J. R. *Biochem. Biophys. Res. Commun.* **1977**, *77*, 387.
(35) Ishizu, K.; Dearman, H. H.; Huang, M. T.; White, J. R. *Biochim. Biophys. Acta* **1968**, *165*, 283.
(36) White, J. R.; Dearman, H. H. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *54*, 887.
(37) White, H. L.; White, J. R. *Mol. Pharmacol.* **1968**, *4*, 549.
(38) Kremer, W. B.; Laszlo, J. *Handb. Exp. Pharmakol.* **1975**, *38*(2), 633.
(39) White, H. L.; White, J. R. *Biochim. Biophys. Acta* **1966**, *123*, 648.
(40) Shaikh, I. A.; Johnson, F.; Grollman, A. P. *J. Med. Chem.* **1986**, *29*, 1329.
(41) For early descriptions of the reversible and irreversible binding of streptonigrin to DNA, cf.: Mizuno, N. S.; Gilboe, D. P. *Biochim. Biophys. Acta* **1970**, *224*, 319. Dudnik, Yu. V.; Gauze, G. G.; Karpov, V. L.; Kozmyan, L. I.; Padron, E. *Antibiotiki (Moscow)* **1973**, *18*, 968; *Chem. Abstr.* **1974**, *80*, 105000e.

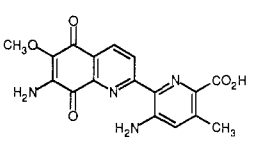
The streptonigrin-induced cell-free, single-strand cleavage of covalently closed circular DNA (ccc-DNA)[30-32] (1) requires an apparent, in situ reduction (NADH activation,[28-38] presumably AB quinone → AB hydroquinone/semiquinone radical), (2) is facilitated by the presence of metal cations including Cu(II) and Fe(II),[30-34,40] (3) is inhibited completely by the addition of ethylenediaminetetraacetic acid (EDTA),[30-33,40] (4) requires the presence and activation of molecular oxygen,[30-40] and (5) is inhibited by superoxide oxidoreductase (E.C. 1.15.1.1) *and* hydrogen peroxide oxidoreductase (E.C. 1.11.1.6).[30-33] Conflicting reports of direct and indirect experimental evidence that suggest the (lack of) association of streptonigrin with double-stranded DNA in the (absence) presence of divalent metal cations continue to cloud the potential mechanism for streptonigrin-induced DNA cleavage.[28-30,34,41-43]

Consequently, two distinct mechanisms have been advanced to accommodate the streptonigrin-induced single-strand cleavage of DNA, streptonigrin cellular toxicity, and presumed mechanism of antitumor activity: (1) the direct participation of streptonigrin as its hydroquinone in the reductive generation of free, diffusible hydroxyl radical (•OH) from molecular oxygen proximal[34] or distal[30-32] to DNA; (2) the direct, covalent interaction of the intermediate streptonigrin semiquinone radical with double-stranded DNA.[28,29]

Limited studies employing synthetic derivatives of streptonigrin[12,13,46-49] have defined the structural components required (7-aminoquinoline-5,8-dione, 7-amino-6-methoxyquinoline-5,8-dione) for in vitro and in vivo cytotoxic, antitumor, or antimicrobial activity as well as those that appear to potentiate (C-6′ carboxylic acid, C-3′ amine) the activity of the naturally occurring agent and that led Rao to propose **3** as the potent, active pharmacophore of the naturally occurring material.[12] Extensive investiga-



3



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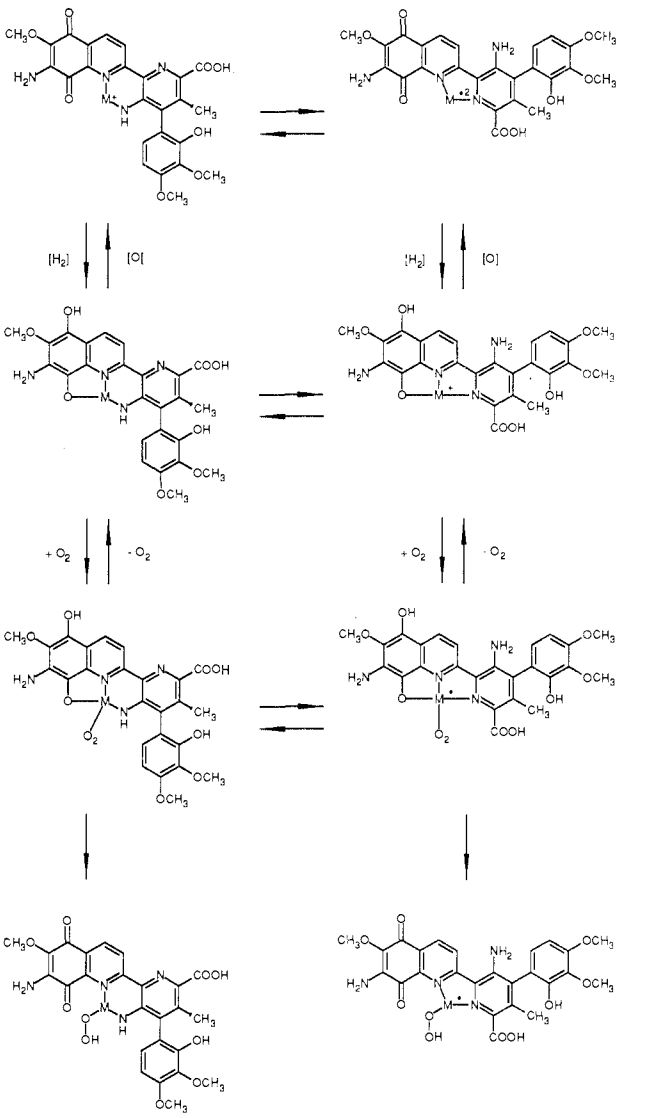
tions focusing on the preparation and evaluation of streptonigrin partial structures[12,13,33,50-54] including the

Scheme I



preparation of 4,[52] lavendamycin partial structures,[53-55] as well as simple, substituted quinoline-5,8-diones[12,13,33,40,50-57] or heterocyclic-fused *p*-benzoquinones[40,56] have confirmed and defined the cytotoxic, antimicrobial, and potential antitumor properties of simplified quinone systems. In selected instances, an excellent correlation of the reduction

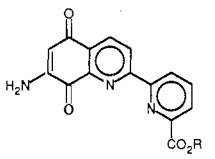
(42) Streptonigrin forms a streptonigrin–Zn(II)–DNA ternary complex (1:7:25 nucleotides) stable to dialysis and gel filtration chromatography; cf. Rao, K. V. *J. Pharm. Sci.* **1979**, *68*, 853.
(43) Hajdu, J.; Armstrong, E. C. *J. Am. Chem. Soc.* **1981**, *103*, 232.
(44) Isopropylazastreptonigrin: Kremer, W. B.; Laszlo, J. *Cancer Chemother. Rep.* **1967**, *51*, 19. Mizuno, N. S. *Biochem. Pharmacol.* **1967**, *16*, 933.
(45) Streptonigrin methyl ester: Kremer, W. B.; Laszlo, J. *Biochem. Pharmacol.* **1966**, *15*, 1111. See also ref 7–13.
(46) Streptonigrin hydrazide, streptonigrin amide(s), streptonigrin methyl ester: Inouye, Y.; Okada, H.; Roy, S. K.; Miyasaka, T.; Hibino, S.; Tanaka, N.; Nakamura, S. *J. Antibiot.* **1985**, *38*, 1429.
(47) Streptonigrone: Herlt, A. J.; Rickards, R. W.; Wu, J.-P. *J. Antibiot.* **1985**, *38*, 516.
(48) 6-*O*-Demethylstreptonigrin: Cohen, M. M.; Shaw, M. W.; Craig, A. P. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *50*, 16.
(49) 10′-*O*-Demethylstreptonigrin: Isshiki, K.; Sawa, T.; Miura, K.; Li, B.; Naganawa, H.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1986**, *39*, 1013.
(50) Streptonigrin AB ring system: Liao, T. K.; Nyberg, W. H.; Cheng, C. C. *J. Heterocycl. Chem.* **1976**, *13*, 1063. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 82.
(51) Streptonigrin CD ring system (inactive): Liao, T. K.; Wittek, P. J.; Cheng, C. C. *J. Heterocycl. Chem.* **1976**, *13*, 1283. *J. Org. Chem.* **1979**, *44*, 870.
(52) Streptonigrin ABC ring system: (a) Rao, K. V. *J. Heterocycl. Chem.* **1977**, *14*, 653. (b) Rao, K. V.; Kuo, H.-S. *J. Heterocycl. Chem.* **1979**, *16*, 1241. (c) Rao, K. V.; Venkateswarlu, P. *J. Heterocycl. Chem.* **1975**, *12*, 731. (d) Rao, K. V. *J. Heterocycl. Chem.* **1975**, *12*, 725. (e) Compound 4, 7-amino-6-methoxy-2-(3′-amino-5′-methyl-2′-pyridyl)-5,8-dioxoquinoline-6′-carboxylic acid, was described to be 2 times as potent as streptonigrin in an in vitro antimicrobial assay against *B. subtilis*; cf. ref 52a.
(53) Streptonigrin and lavendamycin AB and ABC ring systems: Boger, D. L.; Yasuda, M. *Heterocycles* **1986**, *24*, 1067.
(54) Streptonigrin ABCD ring system: Kende, A. S.; Naegely, P. C. *Tetrahedron Lett.* **1978**, 4775.
(55) Lavendamycin AB, ABC, and CDE β-carboline ring systems: (a) Boger, D. L.; Duff, S. R.; Panek, J. S.; Yasuda, M. *J. Org. Chem.* **1985**, *50*, 5782. (b) Boger, D. L.; Panek, J. S. *Tetrahedron Lett.* **1984**, *25*, 3175.
(56) Renault, J.; Giorgi-Renault, S.; Baron, M.; Mailliet, P.; Paoletti, C.; Cros, S.; Viosin, E. *J. Med. Chem.* **1983**, *26*, 1715. Renault, J.; Giorgi-Renault, S.; Mailliet, P.; Baron, M.; Paoletti, C.; Cros, S. *Eur. J. Med. Chem.—Chim. Ther.* **1981**, *16*, 24; **1981**, *16*, 545; **1983**, *18*, 134.
(57) Hodnett, E. M.; Wongwiechintana, C.; Dunn, W. J., III; Marrs, P. *J. Med. Chem.* **1983**, *26*, 570 and references cited therein.

potential of the quinone system and the extent of cell-free single-strand DNA cleavage has been observed.[32,40] None, however, have been described to possess the comparable cytotoxic, antimicrobial, or antitumor potency of streptonigrin despite the enhanced or comparable redox properties.

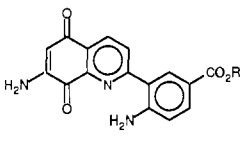
The effective ability of 8-hydroxyquinolines (cf. streptonigrin → streptonigrin semiquinone radical or hydroquinone) to complex divalent metal cations[58] and the feasible potentiation of a streptonigrin hydroquinone (8-hydroxyquinoline) metal chelation, oxygen activation process by the streptonigrin pyridyl N-1′/C-6′ carboxylate *or* acidic C-3′ pyridyl amine have provided an attractive rationale for the observed cytotoxic, antimicrobial, and antitumor potency as well as cell-free single-strand DNA cleavage efficacy of streptonigrin (Scheme I). An attractive feature of such a mechanism of metal cation complexation and molecular oxygen activation has been the direct ligand (streptonigrin hydroquinone) participation (oxidation) in the activation (reduction) of molecular oxygen. The streptonigrin hydroquinone may be reoxidized to the corresponding quinone by stepwise, single-electron or two-electron oxidation concurrent with the reduction of molecular oxygen to a metal peroxo or hydroperoxide species. This species may serve as a powerful oxidizing agent in its own right or as an intermediate en route to the generation of free, diffusible hydroxyl radical (•OH). Consequently, the functional groups capable of facilitating the quinoline-5,8-dione metal complexation have been implicated as being responsible for potentiating the single-strand DNA cleavage capabilities of the agents and responsible for the enhanced cytotoxic, antitumor, and antimicrobial properties of streptonigrin.

In addition, the well-established potentiation of the cytotoxic, antimicrobial, and antitumor properties which may be attributed to the streptonigrin C-6′ carboxylic acid [CO_2H (1a) ≫ CO_2CH_3 (**1b**) ~ $CONHNH_2$ (**1c**)],[45-47] the recognized metal complexation properties of related 2,2′-bipyridyl systems,[59,60] as well as the recent demonstration of the affinity,[61,62] specificity,[61-63] and nuclease oxidative cleavage of native and synthetic polynucleotides promoted by 1,10-phenanthroline metal complexes[61] suggested indirectly that it may be the streptonigrin/lavendamycin C-ring pyridyl N-1′/C-6′ carboxylate that is responsible for the enhanced efficacy of the naturally occurring materials.

Herein we detail the preparation and evaluation of 7-amino-5,8-dioxo-2-(2′-pyridyl)quinoline-6′-carboxylic acid (**5a**) and 7-amino-2-(2′-aminophenyl)-5,8-dioxoquinoline-5′-carboxylic acid (**6a**) required to define the role the streptonigrin/lavendamycin N-1′/C-6′ carboxylate and the acidic, streptonigrin C-3′ pyridyl amine may play in the potentiation of the cytotoxic properties of the 7-aminoquinoline-5,8-dione ring system. An additional, careful comparison of the antimicrobial and cytotoxic properties

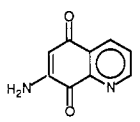


5a, R = H
5b, R = CH_3



6a, R = H
6b, R = CH_3

of a complete set of streptonigrin and lavendamycin partial structures is detailed (**7–13**) in efforts to define the role peripheral substituents play in potentiating the biological properties of the naturally occurring agents bearing the 7-aminoquinoline-5,8-dione AB ring system and in efforts to define the minimum, potent pharmacophore of the naturally occurring antitumor antibiotics. The relationship of these observations to a chemical mechanism of cellular toxicity derived from the oxidative cleavage of double-stranded DNA is discussed.



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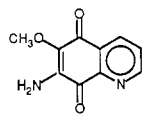

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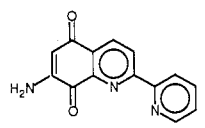

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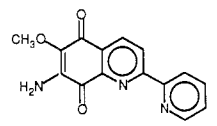

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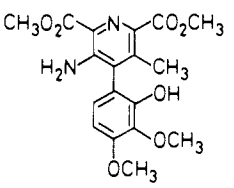

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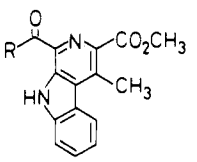
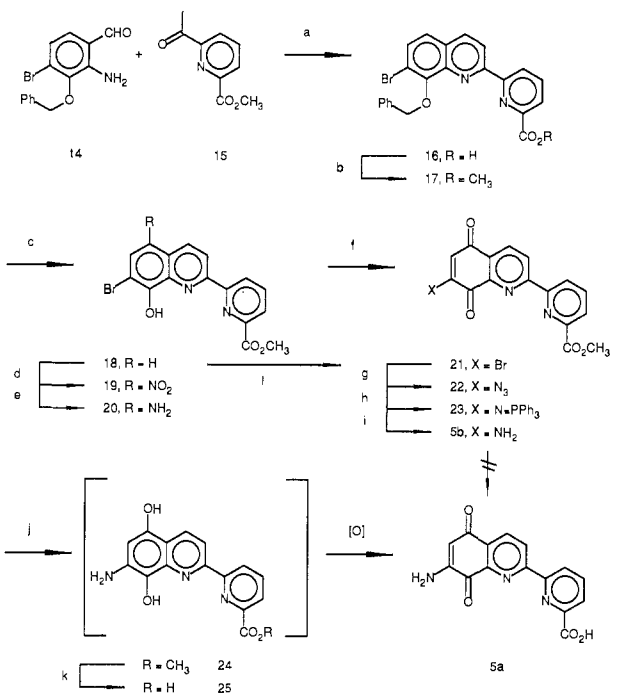


12 R = OCH_3
13 R = CH_3

Preparation of 7-Amino-5,8-dioxo-2-(2′-pyridyl)-quinoline-6′-carboxylic Acid (5a). The preparation of 7-amino-5,8-dioxo-2-(2′-pyridyl)quinoline-6′-carboxylic acid (**5a**) was based on the application of the Friedlander condensation[64] of 2-amino-3-(benzyloxy)-4-bromobenzaldehyde (14)[55a] with methyl 2-acetylpyridine-6-carboxylate (**15**)[65a] for quinoline introduction and assemblage of the

(58) Albert, A. *Selective Toxicity*, 4th ed.; Chapman and Hall: London, England, 1973.
(59) Hay, R. W.; Clark, C. R. *J. Chem. Soc., Dalton Trans.* **1977**, 1866. The log *K* formation (K_F) for Cu(II) complexation with 2-(methoxycarbonyl)-8-hydroxyquinoline is >2 × 10^8 (K_F 8-hydroxyquinoline = 5 × 10^{12}), and metal–carbonyl complexation was observed indirectly.
(60) Creighton, D. J.; Hajdu, J.; Sigman, D. S. *J. Am. Chem. Soc.* **1976**, *98*, 4619.
(61) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180.
(62) Kumar, C. V.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1985**, *107*, 5518.
(63) Barton, J. K.; Raphael, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 6460.
(64) Cheng, C. C.; Yan, S. J. *Org. React. (N.Y.)* **1982**, *28*, 37.
(65) (a) Methyl 2-acetylpyridine-6-carboxylate (**15**) was prepared from pyridine-2,6-dicarboxylic acid as follows: CH_3OH, cat. concentrated H_2SO_4, reflux, 2 days, 96% (dimethyl pyridine-2,6-dicarboxylate); 1.0 equiv of LiOH, THF–CH_3OH–H_2O (3:2:1), 25 °C, 26 h, 34% [2-(methoxycarbonyl)pyridine-6-carboxylic acid]; 2.5 equiv of $(COCl)_2$, cat. DMF, THF, 25 °C, 10 h; 1.3 equiv of $(CH_3)_2CuLi$, THF–Et_2O, −78 °C, 1.5 h, 68%. For **15**: ^1H NMR (200 MHz, $CDCl_3$) δ 8.27 (1 H, dd, *J* = 7.7, 1.2 Hz, C-5H), 8.21 (1 H, dd, *J* = 7.7, 1.2 Hz, C-3H), 7.98 (1 H, t, *J* = 7.7, 7.7 Hz, C-4H), 4.03 (3 H, s, CO_2CH_3), 2.80 (3 H, s, $COCH_3$). (b) Methyl 4-amino-3-acetylbenzoate was prepared by the reaction of methyl 4-aminobenzoate with acetonitrile [1.1 equiv of BCl_3, benzene–CH_2Cl_2 (10:7), reflux, 2 h; 6 equiv of acetonitrile, 1.1 equiv of $AlCl_3$, reflux, 24 h] following the procedure of Sugasawa et al.: Sugasawa, T.; Toyoda, T.; Adachi, M.; Sasakura, K. *J. Am. Chem. Soc.* **1978**, *100*, 4842. For **26**: ^1H NMR (80 MHz, $CDCl_3$) δ 8.47 (1 H, d, *J* = 1.9 Hz, C-2H), 7.91 (1 H, dd, *J* = 8.7, 1.9 Hz, C-6H), 6.74 (2 H, br s, NH_2), 6.63 (1 H, d, *J* = 8.7 Hz, C-5H), 3.88 (3 H, s, CO_2CH_3), 2.63 (3 H, s, $COCH_3$).

Scheme II[a]



[a] (a) Triton B [$PhCH_2(Me)_3NOH$] (4.0 equiv), THF, 0–25 °C, 4 h, 98%. (b) HCl–CH_3OH (10%), 25 °C, 18 h, 85%. (c) HBr(g), CH_2Cl_2, 60 °C, 10 h, 97%. (d) HNO_3 (5.0 equiv), CH_3NO_2, 0 °C, 1 h. (e) Al(Hg), THF–H_2O (10:1), 0 °C, 6 min. (f) MnO_2 (5.0 equiv), aqueous H_2SO_4, 0 °C, 10 min, 33% from 18. (g) NaN_3 (1.1 equiv), THF–H_2O, 25 °C, 21 h, 89%. (h) Ph_3P (1.0 equiv), CH_2Cl_2, 25 °C, 10 min. (i) HOAc–H_2O–THF (3:2:3), 25 °C, 12 min, 56% from 22. (j) $Na_2S_2O_4$ (1.05 equiv), THF–H_2O (1:1), 25 °C, 0.5 h. (k) KOH (7.0 equiv), 25 °C, 1 h, 90% from 5b. (l) $^{\bullet}ON(SO_3K)_2$ (20 equiv), acetone–CH_3OH, 0.05 M KH_2PO_4, 25 °C, 5 h, 32%.

carbon framework of **5a**. The selection of **14** for use in the Friedlander condensation represents one in which the 2-aminobenzaldehyde possesses suitable functionality for 7-aminoquinoline-5,8-dione[55] or 7-amino-6-methoxyquinoline-5,8-dione[53] introduction and one in which the Friedlander condensation[64] could be anticipated to proceed in good conversion.

Consistent with expectations, condensation of 2-amino-3-(benzyloxy)-4-bromobenzaldehyde (**14**)[55a] with methyl 2-acetylpyridine-6-carboxylate[65a] [**15**, 4.0 equiv of Triton B (*N*-benzyltrimethylammonium hydroxide),[66] tetrahydrofuran, 0 °C (1 h), 25 °C (3 h), 94–98%] provided 8-(benzyloxy)-7-bromo-2-(2′-pyridyl)quinoline-6′-carboxylic acid (**16**) directly as the free carboxylic acid (Scheme II). In situ methyl ester hydrolysis promoted by *N*-benzyltrimethylammonium hydroxide (Triton B) proved competitive with the base-promoted Friedlander condensation. Efforts to effect the condensation of **14** with **15** by employing limiting quantities of base (Triton B, 1.0 equiv) or alternative conditions customarily employed to promote a Friedlander condensation failed to provide the methyl ester **17** in competitive conversions.[66b] Fischer esterification of **16** (10% HCl, methanol, 25 °C, 18 h, 85%) followed by O-debenzylation of **17** (HBr(g), CH_2Cl_2, 60 °C, 7 h, 94–97%)[33] provided methyl 7-bromo-8-hydroxy-2-(2′-pyridyl)quinoline-6′-carboxylate (**18**).

Initial, extensive efforts to effect the direct oxidation of phenol **18** to the 7-bromoquinoline-5,8-dione **21** by employing conventional[52,67,68] and recent variants[69] of a Fremy's salt (potassium nitrosodisulfonate)[67] oxidation proved modestly successful. As a result of the modest conversion and the erratic nature (0–30%) of the direct oxidation of **18** under the best conditions devised coupled with the failure of alternative, direct oxidation procedures [Ce$(NH_4)_2(NO_3)_6$, NH_4NO_3–Ac_2O/TFAA][70,71] to provide the 7-bromoquinoline-5,8-dione **21**, an alternative three-step procedure for 7-bromoquinoline-5,8-dione introduction was investigated and implemented. Nitration of the free phenol **18** provided the exceptionally insoluble nitrophenol **19**, and subsequent, sequential aluminum amalgam reduction[72] and manganese dioxide oxidation[73] provided the reactive 7-bromoquinoline-5,8-dione **21** as a yellow, crystalline solid. The combination of aluminum amalgam–manganese dioxide in the reduction (**19** → **20**) oxidation (**20** → **21**) sequence detailed in Scheme II proved more satisfactory and more convenient than efforts employing a sodium dithionite reduction and those using a potassium nitrosodisulfonate or chromic acid oxidation.

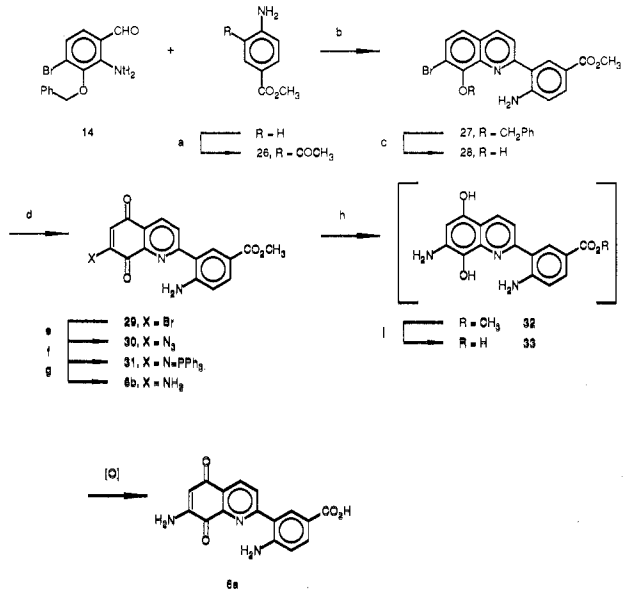
Conversion of 7-bromoquinoline-5,8-dione **21** to the corresponding 7-aminoquinoline-5,8-dione followed the procedure previously developed in efforts on the total synthesis of lavendamycin.[28,55] Direct C-7 sodium azide displacement of the 7-bromoquinoline-5,8-dione under carefully controlled experimental conditions provided the 7-azidoquinoline-5,8-dione **22** in a reaction that proved sensitive to the presence of excess sodium azide.[55a] Azide reduction, which was most effectively accomplished by employing triphenylphosphine,[55a] provided methyl 7-amino-5,8-dioxo-2-(2′-pyridyl)quinoline-6′-carboxylate (**5b**) in a reduction that proceeds without competitive quinoline-5,8-dione reduction and with the intermediacy of the stable, characterizable triphenylphosphine imide **23**. Efforts to promote the methyl ester hydrolysis of **5b** to provide **5a** directly were unsuccessful due to predominant and competitive nucleophilic addition to the AB quinoline-5,8-dione system. In situ reduction of the quinoline-5,8-dione to the corresponding hydroquinone **24** followed by base-promoted methyl ester hydrolysis provided the desired carboxylic acid **5a**. Oxidation of hydroquinone **25** to quinoline-5,8-dione **5a** occurs upon workup and exposure to air.

Preparation of 7-Amino-2-(2′-aminophenyl)-5,8-dioxoquinoline-5′-carboxylic Acid (6a). Friedlander condensation[64] of methyl 3-acetyl-4-aminobenzoate (**26**)[65b] with 2-amino-3-(benzyloxy)-4-bromobenzaldehyde (**14**)[55] provided the quinoline **27** [4.0 equiv of benzyltrimethylammonium hydroxide (Triton B),[66] tetrahydrofuran, 0–25 °C, 6 h, 81%] (Scheme III). Debenzylation of **27**, which was most effectively accomplished with anhydrous hydrogen bromide in methylene chloride, provided the free phenol **28** (87%). After a number of unsuccessful attempts,[52a,67-71] the direct Fremy's salt (potassium nitrosodisulfonate) oxidation of phenol **28** employing the two-

(66) (a) Available from Aldrich Chemical Co. (b) When 2.0 equiv of sodium methoxide was used (THF, 25 °C, 0.7 h), the Friedlander condensation of **15** with **14** provided **17** (60% optimized).

(67) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.
(68) Hibino, S.; Weinreb, S. M. *J. Org. Chem.* **1977**, *42*, 232.
(69) Kende, A. S.; Ebetino, F. H. *Tetrahedron Lett.* **1984**, *25*, 923. Kende, A. S.; Ebetino, F. H.; Battista, R.; Lorah, D. P.; Lodge, E. *Heterocycles* **1984**, *21*, 91.
(70) Hibino, S.; Okazaki, M.; Ichikawa, M.; Sato, K.; Ishizu, T. *Heterocycles* **1985**, *23*, 261.
(71) Crivello, J. V. *J. Org. Chem.* **1981**, *46*, 3056.
(72) Corey, E. J.; Chaykovsky, M. J. *J. Am. Chem. Soc.* **1965**, *87*, 1345. Meyers, A. I.; Durandetta, J. L.; Marava, R. *J. Org. Chem.* **1975**, *40*, 2025.
(73) MacKenzie, A. R.; Moody, C. J.; Rees, C. W. *Tetrahedron* **1986**, *42*, 3259.

Scheme III[a]



[a] (a) Reference 65b. (b) Triton B [$PhCH_2(Me)_3NOH$] (4.0 equiv), THF, 0–25 °C, 6 h, 81%. (c) HBr(g), CH_2Cl_2, 60 °C, 10 h, 87%. (d) $^{\cdot}ON(SO_3K)_2$ (10 equiv), 1:1 CH_2Cl_2–1.0 M phosphate buffer (KH_2PO_4/Na_2HPO_4 = 1), 1.0 equiv of $(n\text{-}Bu)_4NHSO_4$, 25 °C, 20 h. (e) NaN_3 (1.0 equiv), THF–H_2O, 25 °C, 0.5 h (89% overall from **28**). (f) Ph_3P (1.0 equiv), CH_2Cl_2–MeOH, 25 °C, 1.5 h, 65%. (g) HOAc–H_2O–THF (3:2:3), 25 °C, 0.2 h, 99%. (h) $Na_2S_2O_4$ (5.0 equiv), THF–MeOH–H_2O (3:2:1), 25 °C, 0.5 h. (i) LiOH (10 equiv), 50 °C, 35 h, 66% from **6b**.

phase oxidation conditions introduced by Kende[69] provided the exceptionally insoluble and unstable 7-bromoquinoline-5,8-dione **29**. The insoluble and unstable nature of the 7-bromoquinoline-5,8-dione **29** precluded conventional purification. The conversion of the crude 7-bromoquinoline-5,8-dione **29** to the corresponding 7-aminoquinoline-5,8-dione followed the procedure developed in conjunction with efforts on the total synthesis of lavendamycin.[28,55] Direct C-7 sodium azide displacement employing the crude 7-bromoquinoline-5,8-dione under carefully controlled experimental conditions provided the stable, soluble 7-azidoquinoline-5,8-dione **30**. Azide reduction, which was most effectively accomplished by employing triphenylphosphine,[28c,55a] provided methyl 7-amino-2-(2′-aminophenyl)-5,8-dioxoquinoline-5′-carboxylate (**6b**) in a reduction that proceeds without competitive quinoline-5,8-dione reduction and with the intermediacy of the stable, characterizable triphenylphosphine imide **31**. In situ reduction of the quinone **6b** to the corresponding hydroquinone **32** followed by base-promoted methyl ester hydrolysis provided the desired carboxylic acid **6a**. Oxidation of the hydroquinone **33** to the 7-aminoquinoline-5,8-dione **6a** occurs upon workup and exposure to air.

Biological Results and Discussion

The in vitro antimicrobial assays were performed with an agar dilution/streak assay[74] against seven microorganisms: *Staphylococcus aureus* ATCC 13709, *Escherichia coli* ATCC 9637, *Salmonella gallinarum* ATCC 9184, *Klebsiella pneumoniae* ATCC 10031, *Mycobacterium smegmatis* ATCC 607, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231. The results (MIC, μg/mL) are detailed in Table I. The in vitro cytotoxic: IC_{50},[b,c] μg/mL

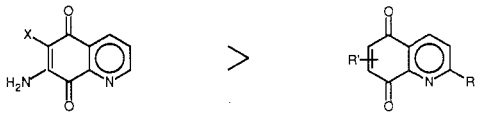
Table I. In Vitro Antimicrobial and Cytotoxic Activity

	antimicrobial: MIC,[a,b] μg/mL							cytotoxic: IC_{50},[b,c] μg/mL				
	S. aureus ATCC 13709	*E. coli* ATCC 9637	*S. gallinarum* ATCC 9184	*K. pneumoniae* ATCC 10031	*M. smegmatis* ATCC 607	*C. albicans* ATCC 10231	*P. aeruginosa* ATCC 27853	CCRF-CEM[d]	L-1210[e]	B16[f]	9PS (P388)[g]	9KB[h]
1a	<0.1	6.25	1.56	0.1	1.56	12.5	6.25	0.00017	0.61	0.48	0.045	0.0025
1b	>100	>100	>100	>100	>100	>100	>100	0.017	3.1	1.5	0.26	0.53
2a	(0.16)[18]	(1)		(>125)		(16)	(16)		0.9	1.1		
2b									2.2	3.1		
5a	>50	>50	>50	>50	>50	>50	>50	2.1	>20	>20	>10	>10
5b	6.25	>50	>50	50	6.25	>50	>50	0.1	0.4	0.9	3.9	0.17
6a	>50	>50	>50	>50	>50	>50	>50	14.6	>20	>20	5.0	>10
6b	>50	>50	>50	>50	>50	>50	>50	0.4	0.6	0.4	0.2	2.7
7	12.5	50	25	50	12.5	50	>100	0.3	0.34	0.14	0.19	0.74
8	6.25	>50	>50	50	50	50	>50	0.2	1.0	1.2	0.22	1.9
9	3.12	>100	100	100	0.78	50	>100	0.09	0.42	0.26	0.0023	0.10
10	1.56	>100	>100	25	1.56	3.12	>100	0.5	0.30	0.45	0.034	0.33
11	>50	>50	>50	>50	>50	>50	>50		>20	>20	>10	>10
12	>50	>50	>50	>50	50	>50	>50	16.8	>25	>25		
13	>50	>50	>50	>50	>50	>50	>50		>25	>25		

[a] Minimum inhibitory concentration (MIC). [b] American Type Culture Collection (ATCC). [c] Inhibitory concentration for 50% cell growth relative to untreated control (IC_{50}). [d] Human lymphoblastic leukemia cell culture.[75] [e] L-1210 mouse lymphocytic leukemia cell culture.[77] [f] B16 mouse melanoma cell culture.[78] [g] P388 mouse leukemia cell culture.[78] [h] Human epidermoid carcinoma of the nasopharynx.[78]

(74) Mitscher, L. A.; Leu, R.-P.; Bathala, M. S.; Wu, W.-N.; Beal, J. L.; White, R. *Lloydia* **1972**, *35*, 157.

totoxic assays were performed by using five cell culture assays: CCRF-CEM (human lymphoblastic leukemia),[75] B16 (mouse melanoma),[76,77] L-1210 (mouse lymphocytic leukemia),[77] 9PS (P388 mouse leukemia),[78] and 9KB (human epidermoid carcinoma of the nasopharynx).[78] The results, inhibitory concentration for 50% cell growth relative to untreated controls (IC_{50}, $\mu g/mL$), are detailed in Table I. In initial, direct comparisons of antimicrobial and cytotoxic properties of the synthetic quinoline-5,8-diones, the 7-aminoquinoline-5,8-dione ring system (lavendamycin AB ring system) proved more potent than the 7-amino-6-methoxyquinoline-5,8-dione ring system (streptonigrin AB ring system) [Table I: **7** vs. **8**; **9** vs. **10**]. Consistent with past observations, both the 7-aminoquinoline-5,8-dione and 7-amino-6-methoxyquinoline-5,8-dione ring systems proved substantially more potent than alternative, substituted quinoline-5,8-diones including the 6-aminoquinoline-5,8-dione ring system [Table II, supplementary material]. Contrary to initial expectations, the results would suggest that the enhanced potency of streptonigrin (1a) vs. lavendamycin (**2a**) is not due to differences in intrinsic potency of the AB quinoline-5,8-dione ring systems but to a substantial potentiation of the cytotoxic properties attributable to the peripheral streptonigrin CD vs. lavendamycin CDE ring systems.



X = H > OCH_3

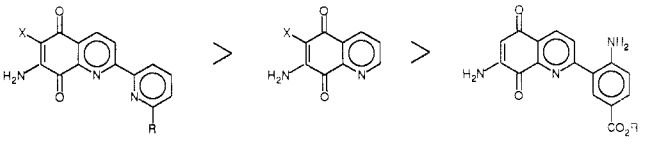
The fully elaborated streptonigrin CD and lavendamycin CDE ring systems (Table I, **11** and **12**, **13** respectively) as well as a number of related synthetic structures (Table II, supplementary material) proved inactive in the antimicrobial and cytotoxic assays. The observed lack of activity with the intact streptonigrin CD ring system **11** has been detailed in the early investigations of Cheng.[51]

Streptonigrin (1a) proved substantially more potent than streptonigrin methyl ester (**1b**) as previously detailed[45-47] as well as lavendamycin (**2a**)[25] and lavendamycin methyl ester (**2b**).

Evaluation of the synthetic agents bearing only the streptonigrin C-ring N-1′ pyridyl nitrogen provided by introduction of the 2-(2′-pyridyl) unit onto the 7-aminoquinoline-5,8-dione or 7-amino-6-methoxyquinoline-5,8-dione ring system provided agents with increased cytotoxic potency (Table I, **9** vs. **7**; **10** vs. **8**). 7-Amino-2-(2′-pyridyl)quinoline-5,8-dione (**9**) proved to be an agent comparable in cytotoxic potency to streptonigrin albeit with altered selectivity. Streptonigrin was found to be exceptionally potent in the CCRF-CEM and 9KB cell culture assays (IC_{50} = 0.17 ng/mL and 2.5 ng/mL respectively) while **9** displayed potent cytotoxic activity in the 9PS (P388) cell culture assay (IC_{50} = 2.3 ng/mL).

Evaluation of the synthetic agents **5a,b** provided the opportunity to probe the role the streptonigrin C-ring N-1′/C-6′ carboxylate may play in effecting the streptonigrin antimicrobial/cytotoxic activity. The cytotoxic properties of 7-amino-5,8-dioxo-2-(2′-pyridyl)quinoline-6′-carboxylate as its methyl ester **5b** proved comparable to the parent 7-amino-2-(2′-pyridyl)quinoline-5,8-dione (**9**) with the exception of a marked decrease in 9PS (P388) cytotoxic activity. A loss of antimicrobial and cytotoxic activity was observed with the free carboxylic acid derivative **5a** (inactive) which accompanied the introduction of the C-6′ carboxylic acid onto the potent, parent 7-amino-2-(2′-pyridyl)quinoline-5,8-dione. Thus, in contrast to observations associated with streptonigrin in which the C-6′ carboxylic acid potentiates the antitumor, antimicrobial, and cytotoxic activity of the naturally occurring quinoline-5,8-dione, the C-6′ carboxylic acid of **5a** diminishes the observed antimicrobial and cytotoxic properties of the parent 7-amino-2-(2′-pyridyl)quinoline-5,8-dione. Consequently, these comparative results would suggest that the streptonigrin C-ring N-1′/C-6′ carboxylate does not potentiate the properties of a 7-aminoquinoline-5,8-dione by a productive participation of the C-6′ carboxylic acid in metal complexation.

Evaluation of the synthetic agents **6a,b** (lacking the streptonigrin C-ring N-1′ pyridyl nitrogen) provided the opportunity to probe the role the streptonigrin C-ring C-3′ pyridyl amine/C-6′ carboxylate may play in potentiating the antimicrobial/cytotoxic properties of streptonigrin. 7-Amino-2-(2′-aminophenyl)-5,8-dioxoquinoline-5′-carboxylate as its methyl ester **6b** proved less potent than the parent 7-aminoquinoline-5,8-dione (Table I: **6b** vs. **7**) and substantially less potent than the 7-amino-2-(2′-pyridyl)quinoline-5,8-dione systems bearing the streptonigrin C-ring N-1′ nitrogen (Table I: **6b** vs. **5b**, **9**, **10**). A loss of antimicrobial and cytotoxic activity was observed with the free carboxylic acid **6a**. Thus, in contrast to the observations on streptonigrin, the introduction of the free carboxylic acid further diminished the cytotoxic properties of the parent 7-aminoquinoline-5,8-dione (Table I: **6a** vs. **7**) and abolished the marginal cytotoxic properties of the 7-amino-2-(2′-aminophenyl)quinoline-5,8-dione system (**6a** vs. **6b**).



A B C

A: R = H (X = H > OCH_3) ≳ CO_2CH_3 (X = H) ≫ CO_2H (X = H, inactive)

B: X = H > OCH_3

C: R = CH_3 ≫ H (inactive)

A comparison of the results of the antimicrobial and cytotoxic properties of the synthetic streptonigrin and lavendamycin partial structures suggests a prominent role for the streptonigrin C-ring N-1′ nitrogen in potentiating the cytotoxic potency of the 7-aminoquinoline-5,8-dione/7-amino-6-methoxyquinoline-5,8-dione ring system and a minimal, direct participation in the potentiation of the properties by the streptonigrin C-ring C-3′ pyridyl amine/C-6′ carboxylate. While the mechanism of streptonigrin cellular toxicity may require metal complexation and a subsequent role in oxygen activation, the potentiation of the antimicrobial and cytotoxic properties of the streptonigrin/lavendamycin 7-aminoquinoline-5,8-dione AB ring system is not due to enhanced metal complexation properties introduced by the streptonigrin peripheral C-ring substituents. Moreover, the unrecognized origin of the well-established potentiation of the cytotoxic, antimicrobial, and antitumor properties of streptonigrin which

(75) Foley, G. E.; Lazarus, H.; Farber, S.; Uzman, B. G.; Boone, B. A.; McCarthy, R. E. *Cancer (Philadelphia)* **1965**, *18*, 522. Foley, G. E.; Lazarus, H. *Biochem. Pharmacol.* **1967**, *16*, 659.
(76) Donoso, J. A.; Himes, R. H. *Cancer Biochem. Biophys.* **1984**, *7*, 133.
(77) Boger, D. L.; Mitscher, L. A.; Mullican, M. D.; Drake, S. D.; Kitos, P. A. *J. Med. Chem.* **1985**, *28*, 1543.
(78) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*(2), 17–20, 59–61.

may be attributed to the streptonigrin C-6′ carboxylic acid appears to override diminished intrinsic activity which the addition of this group imparts to structurally related, synthetic 7-aminoquinoline-5,8-diones.

In Vivo Antitumor Evaluation. Three 7-aminoquinoline-5,8-diones, 7-aminoquinoline-5,8-dione (**7**), 7-amino-6-methoxyquinoline-5,8-dione (**8**), and 7-amino-2-(2′-pyridyl)quinoline-5,8-dione (**9**), displaying potent cytotoxic properties have been evaluated for in vivo antitumor activity.[79,80] The results are summarized below along with comparative antitumor testing results on streptonigrin (**1a**),[79] streptonigrin methyl ester (**1b**),[79] and lavendamycin (**2a**)[79,81] [tumor (dose schedule), dose (T/C or tumor inhibition, survivors/number of treated animals)]. Each of the synthetic 7-aminoquinoline-5,8-diones **7–9** proved toxic at doses comparable to those required or expected for observable antitumor activity.

7: 2CA12 (1 × daily/11 days) 15.0/10.0/6.5 mg/kg (toxic, 7/10; toxic, 9/10; 111, 10/10);[79] 6C3HED lymphosarcoma (1 × daily/10 days), 45.0/22.0 mg/kg (13% reduction in tumor size, 10/10; 3% reduction in tumor size, 10/10).[80]

8: 6PS31 leukemia (1 × daily/9 days), 32.0/16.0/8.0/4.0 mg/kg (toxic, 0/6; 102, 6/6; 108, 6/6; 100, 6/6);[79] 3PS31 leukemia (1 × daily/10 days), 32.0/16.0/8.0/4.0 mg/kg (toxic, 0/6; 127, 6/6; 109, 6/6; 113, 6/6).[79]

9: 6C3HED lymphosarcoma (1 × daily/10 days), 7.5/3.75 mg/kg (36% reduction in tumor size, 8/10; 7% reduction in tumor size, 9/10).[80]

1a: 3PS31 leukemia (1 × daily/9 days), 0.8/0.4/0.2/0.16/0.12/0.10/0.09/0.08/0.06/0.05 mg/kg (117, 6/6; toxic and 145, 6/6 and 6/6; 143–123, 8/8 and 6/6; 144, 8/8; 130–119, 8/8 and 8/8; 152, 6/6; 129, 8/8; 117–88, 8/8 and 8/8; 126, 8/8; 161, 6/6);[79] 5WA21 (1 × daily/9 days), 0.8/0.4/0.2/0.1 (toxic, 6/6; 131, 6/6; 453, 6/6; 434, 6/6).[79]

1b: 3PS31 leukemia (1 × daily/9 days), 25.0/12.5/9.0/6.0/4.0 mg/kg (129, 6/6; 137, 6/6; 162, 6/6; 183, 6/6; 250, 6/6).[79]

2a: 6PS31 leukemia (1 × daily/9 days), 3.2/1.6/0.8/0.4/0.2/0.1 mg/kg (toxic, 6/6; toxic, 6/6; 113, 6/6; 112, 6/6; 116, 5/6; 110, 6/6).[79,81]

Experimental Section

Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian FT-80A, Varian XL-200, or Nicolet NT-470 spectrometer, and chemical shifts are reported in parts per million (ppm) relative to internal tetramethylsilane (0.00 ppm). Infrared spectra (IR) were recorded on a Perkin-Elmer 1420 or Perkin-Elmer 1710 Fourier transform spectrometer as KBr pellets. Melting points (mp) were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Electron-impact mass spectra (EIMS) and chemical-ionization mass spectra (CIMS) were recorded on a Varian CH-5 or a Finnegan 4000 spectrometer. High-resolution mass spectra (HRMS) were recorded on a Kratos MS-50 spectrometer. Chromatography was performed on a 60–200-mesh silica gel. Tetrahydrofuran (THF) and ether (Et_2O) were distilled from benzophenone ketyl. Methanol (MeOH) was distilled from magnesium methoxide. *N*,*N*-Dimethylformamide (DMF) was distilled from calcium hydride. Methylene chloride (CH_2Cl_2) was distilled from phosphorus pentoxide. All extraction and chromatographic solvents, ethyl acetate (EtOAc), hexane, and methylene chloride (CH_2Cl_2), were distilled prior to use. Fremy's salt (potassium nitrosodisulfonate) was freshly prepared as detailed[67] or purchased from Aldrich Chemical Co. Activated manganese dioxide (MnO_2) was obtained from Alfa Products and was dried (150 °C, 6–12 h) prior to use. All reactions requiring anhydrous conditions and/or an inert atmosphere were performed under a positive pressure of nitrogen (N_2) or argon.

Streptonigrin (**1a**) was generously provided by the National Institutes of Health, National Cancer Institute. Streptonigrin methyl ester (**1b**) was prepared as detailed.[6a] Lavendamycin (**2a**) and lavendamycin methyl ester (**2b**) were prepared as previously described.[28c] 7-Aminoquinoline-5,8-dione (**7**),[55a] 7-amino-6-methoxyquinoline-5,8-dione (**8**),[53] 7-amino-2-(2′-pyridyl)-quinoline-5,8-dione (**9**),[55a] 7-amino-6-methoxy-2-(2′-pyridyl)-quinoline-5,8-dione (**10**),[53] and the lavendamycin β-carboline CDE ring systems **12**–**13**[55a,b] were prepared as detailed.

Methyl 8-(Benzyloxy)-7-bromo-2-(2′-pyridyl)quinoline-6′-carboxylate (17). A solution of methyl 2-acetylpyridine-6-carboxylate (**15**;[65a] 123 mg, 0.69 mmol, 1.0 equiv) in tetrahydrofuran (7 mL) was treated with a 40% methanolic solution of *N*-benzyltrimethylammonium hydroxide (Triton B,[66] 1.15 g, 2.75 mmol, 4.0 equiv) at 0 °C under N_2. A solution of 2-amino-3-(benzyloxy)-4-bromobenzaldehyde (**14**;[55] 231 mg, 0.76 mmol, 1.1 equiv) in tetrahydrofuran (1.0 mL) was added to the reaction mixture. The reaction mixture was stirred at 0 °C (1 h) and at 25 °C (3 h), diluted with saturated ammonium chloride (5 mL), and further diluted with water (20 mL). The precipitated white material was collected by filtration and washed with water and *n*-hexane, and the solvent was removed in vacuo to afford the carboxylic acid **16** (280 mg, 299 mg theor, 94%, 94–98%) as a white solid: mp 215–216 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 8.78 (1 H, dd, J = 7.6, 1.4 Hz, C-5′H), 8.55 (1 H, d, J = 8.7 Hz, C-4H), 8.33 (1 H, d, J = 8.7 Hz, C-3H), 8.32 (1 H, dd, J = 7.6, 1.4 Hz, C-3′H), 8.05 (1 H, t, J = 7.6, 7.6 Hz, C-4′H), 7.77 (1 H, d, J = 8.9 Hz, C-6H), 7.53 (1 H, d, J = 8.9 Hz, C-5H), 7.30–7.75 (5 H, m, aromatic H), 5.57 (2 H, s, CH_2Ph); IR (KBr) ν_{max} 3400, 3026, 1600, 1578, 1566, 1494, 1431, 1406, 1385, 1371, 1325, 1256, 1215, 1089, 964, 845, 783 cm^{-1}; EIMS, *m*/*e* (relative intensity) 434/436 (M^+, 1/1, 3), 357/359 (1/1, 6); CIMS (NH_3), *m*/*e* (relative intensity) 435/437 (M + 1, 1/1, base); HRMS, *m*/*e* for $C_{22}H_{15}BrN_2O_3$ calcd 434.0266, found 434.0271.

The carboxylic acid **16** (208 mg, 0.48 mmol) was added to a stirred solution of 10% HCl–MeOH (15 mL) at 0 °C. The reaction mixture was stirred at 25 °C (18 h) before the solvent was removed in vacuo. The crude reaction product was dissolved in CH_2Cl_2 (30 mL), washed with water (20 mL) and saturated aqueous NaCl (20 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 1 × 20 cm, 10% EtOAc–hexane eluant) afforded pure **17** (183 mg, 215 mg theor, 85%) as a white solid: mp 130.5–131.5 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 8.77 (1 H, d, J = 8.7 Hz, C-4H), 8.72 (1 H, dd, J = 7.6, 1.3 Hz, C-5′H), 8.28 (1 H, d, J = 8.7 Hz, C-3H), 8.18 (1 H, dd, J = 7.6, 1.3 Hz, C-3′H), 7.93 (1 H, t, J = 7.6, 7.6 Hz, C-4′H), 7.70 (1 H, d, J = 8.8 Hz, C-6H), 7.48 (1 H, d, J = 8.8 Hz, C-5H), 7.35–7.76 (5 H, m, aromatic H), 5.58 (2 H, s, CH_2Ph), 4.05 (3 H, s, CO_2CH_3); IR (KBr) ν_{max} 1733, 1604, 1588, 1496, 1455, 1374, 1323, 1252, 1143, 1091, 836, 727 cm^{-1}; EIMS, *m*/*e* (relative intensity) 448/450 (M^+, 1/1, 5), 371/373 (1/1, 13); CIMS (NH_3), *m*/*e* (relative intensity) 449/451 (M + 1, 1/1, base); HRMS, *m*/*e* for $C_{23}H_{17}BrN_2O_3$ calcd 448.0422, found 448.0427.

Methyl 7-Bromo-8-hydroxy-2-(2′-pyridyl)quinoline-6′-carboxylate (18). A solution of methyl 8-(benzyloxy)-7-bromo-2-(2′-pyridyl)quinoline-6′-carboxylate (**17**; 273 mg, 0.61 mmol) in CH_2Cl_2 (7 mL) saturated with HBr gas was warmed at 60 °C for 10 h in a sealed tube.[82] The reaction mixture was cooled, and saturated $NaHCO_3$ (5 mL) was added with stirring until the

(79) The data are the results of screening performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

(80) The data are the results of screening performed by Eli Lilly and Company. We thank Dr. Homer Pearce, Sharon M. Rinzel, and Carol Katerjohn for providing us with this testing information.

(81) For a report of the full evaluation of the in vivo antitumor activity of lavendamycin (**2a**), see ref 25.

(82) The reaction was run in a 2.0-mL resealable Kontes vial equipped with a Teflon seal/liner (50 mg of **17**, 1.5 mL of CH_2Cl_2) or a 20-mL, thick-walled resealable glass tube internally threaded on one end and sealed under nitrogen with a threaded, solid Teflon plug (273 mg of **17**, 7 mL of CH_2Cl_2; 375 mg of **27**, 10 mL of CH_2Cl_2). The vessels are available from Ace Glass Company.

yellow suspension dissolved completely. The mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. Washing the residue with hexane afforded pure **18** (206 mg, 218 mg theor, 94%, 94–97%) as an off-white solid: mp 197–198 °C; ^{1}H NMR (470 MHz, $CDCl_3$) δ 8.77 (1 H, d, J = 8.6 Hz, C-4H), 8.75 (1 H, d, J = 8.0 Hz, C-5′H), 8.31 (1 H, d, J = 8.6 Hz, C-3H), 8.22 (1 H, d, J = 8.0 Hz, C-3′H), 8.06 (1 H, t, J = 7.8, 7.8 Hz, C-4′H), 7.65 (1 H, d, J = 8.8 Hz, C-6H), 7.30 (1 H, d, J = 8.8 Hz, C-5H), 4.07 (3 H, s, CO_2CH_3); IR (KBr) ν_{max} 3380, 3076, 1732, 1584, 1494, 1460, 1440, 1308, 1247, 1166, 1115, 984, 835, 770 cm^{-1}; EIMS, m/e (relative intensity) 358/360 (M^+, 1/1, 90), 298/300 (1/1, base); CIMS (NH_3), m/e (relative intensity) 359/361 (M + 1, 1/1, base); HRMS, m/e for $C_{16}H_{11}BrN_2O_3$ calcd 357.9953, found 357.9964.

Methyl 7-Bromo-5,8-dioxo-2-(2′-pyridyl)quinoline-6′-carboxylate (21). A stirred suspension of methyl 7-bromo-8-hydroxy-2-(2′-pyridyl)quinoline-6′-carboxylate (**18**; 205 mg, 0.57 mmol) in CH_3NO_2 (10 mL) was treated with a 1 M solution of nitric acid in CH_3NO_2 (2.90 mL, 2.90 mmol, 5 equiv) at 0 °C under a N_2 atmosphere, and the mixture was stirred at 0 °C for 1 h. The reaction mixture was diluted with water (100 mL) and extracted with CH_2Cl_2 (4 × 80 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. Trituration of the crude product with n-hexane afforded methyl 7-bromo-8-hydroxy-5-nitro-2-(2′-pyridyl)quinoline-6′-carboxylate (**19**, 226 mg, 231 mg theor, 98%) as a yellow, insoluble solid: mp 217–218 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 9.44 (1 H, d, J = 9.2 Hz, C-4H), 9.02 (1 H, d, J = 9.2 Hz, C-3H), 8.78 (1 H, s, C-6H), 8.72 (1 H, dd, J = 7.5, 1.3 Hz, C-5′H), 8.29 (1 H, dd, J = 7.5, 1.3 Hz, C-3′H), 8.09 (1 H, t, J = 7.5, 7.5 Hz, C-4′H), 4.08 (3 H, s, CO_2CH_3); EIMS, m/e (relative intensity) 403/405 (M^+, 1/1, base); CIMS (NH_3), m/e (relative intensity) 404/406 (M + 1, 1/1, 72); HRMS, m/e for $C_{16}H_{10}BrN_3O_5$ calcd 402.9804, found 402.9812.

The nitrophenol (**19**; 20 mg, 0.050 mmol) was dissolved in tetrahydrofuran–water (3.3 mL, 10:1) and cooled to 0 °C. The mixture was treated with freshly prepared aluminum amalgam[41] (100 mg, 5 wt equiv) for 6 min at 0 °C. The resulting reaction mixture was filtered through Celite. The Celite was washed with EtOAc (25 mL), and concentration of the filtrate afforded the crude aminophenol **20** (16.3 mg, 0.044 mmol) as a brown solid.

Activated manganese dioxide (19 mg, 0.22 mmol, 5 equiv) was added to a solution of crude **20** (16.3 mg, 0.044 mmol) in aqueous sulfuric acid (35%; 1.5 mL) at 0 °C. The mixture was stirred at 0 °C for 10 min and filtered through Celite. The Celite was washed with water (20 mL) and CH_2Cl_2 (20 mL). The organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed in vacuo. Chromatography (SiO_2, 1 × 11.5 cm, 50% EtOAc–hexane eluant) afforded the pure 7-bromoquinoline-5,8-dione **21** (6.0 mg, 0.016 mmol, 33% from **19**) as a yellow solid: mp 221–222 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 9.02 (1 H, d, J = 8.3 Hz, C-4H), 8.91 (1 H, dd, J = 7.5, 1.5 Hz, C-5′H), 8.54 (1 H, d, J = 8.3 Hz, C-3H), 8.25 (1 H, dd, J = 7.5, 1.5 Hz, C-3′H), 8.05 (1 H, t, J = 7.5, 7.5 Hz, C-4′H), 7.63 (1 H, s, C-6H), 4.05 (3 H, s, CO_2CH_3); IR (KBr) ν_{max} 3057, 1720, 1696, 1664, 1580, 1458, 1434, 1316, 1302, 1294, 1247, 1136, 1119, 964, 842, 807, 745 cm^{-1}; CIMS (NH_3), m/e (relative intensity) 375/377 (M + 2H + 1, base/80), 373/375 (M + 1, 20/base); HRMS, m/e for $C_{16}H_9BrN_2O_4$ calcd 372.9824, found 372.9836.

Repetitive, comparable reactions (0.025–0.050 mmol) provided the 7-bromoquinoline-5,8-dione **21** (24–33% from **19**).

Methyl 7-Azido-5,8-dioxo-2-(2′-pyridyl)quinoline-6′-carboxylate (22). A stirred suspension of methyl 7-bromo-5,8-dioxo-2-(2′-pyridyl)quinoline-6′-carboxylate (**21**; 24 mg, 0.064 mmol) in CH_2Cl_2–H_2O–MeOH (0.65 mL, 9:2:2) was treated with sodium azide (4.6 mg, 0.071 mmol, 1.1 equiv) at 25 °C under a N_2 atmosphere, and the mixture was stirred at 25 °C for 18 h. The reaction mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. Rapid chromatography (SiO_2, 1 × 11 cm, 30% EtOAc–hexane eluant) afforded pure **22** (18.0 mg, 21.6 mg theor, 84%) as a yellow-brown solid: mp 128–129 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 9.00 (1 H, d, J = 8.3 Hz, C-4H), 8.87 (1 H, dd, J = 7.6, 1.5 Hz, C-5′H), 8.54 (1 H, d, J = 8.3 Hz, C-3H), 8.24 (1 H, dd, J = 7.6, 1.5 Hz, C-3′H), 8.04 (1 H, t, J = 7.6, 7.6 Hz, C-4′H), 6.56 (1 H, s, C-6H), 4.05 (3 H, s, CO_2CH_3); IR (KBr) ν_{max} 2120 (N_3), 1735, 1713, 1685, 1642, 1578, 1426, 1348, 1260, 985 cm^{-1}.

Similarly, a stirred suspension of **21** (5.0 mg, 0.013 mmol) in THF–H_2O (0.125 mL, 4:1) was treated with a solution of sodium azide (0.96 mg, 0.015 mmol, 1.1 equiv) in 0.025 mL of water at 25 °C under a N_2 atmosphere and was stirred at 25 °C for 21 h. Chromatography (SiO_2, 1 × 10.5 cm, 30% EtOAc–hexane eluant) afforded pure **22** (4.0 mg, 4.5 mg theor, 89%).

Methyl 7-Amino-5,8-dioxo-2-(2′-pyridyl)quinoline-6′-carboxylate (5b). A stirred solution of the azide **22** (18 mg, 0.054 mmol) in dry CH_2Cl_2 (1 mL) under a N_2 atmosphere was treated with a solution of triphenylphosphine (14.1 mg, 0.054 mmol, 1.0 equiv) in 0.5 mL of CH_2Cl_2 with stirring. Evolution of nitrogen was visible within 1 min after addition of triphenylphosphine. After 10 min at 25 °C the solvent was removed in vacuo. Chromatography (SiO_2, 1 × 9 cm, 40% EtOAc–hexane eluant) afforded the phosphine imide **23** (20 mg, 30.6 mg theor, 65%, 65–68%) as a red solid: mp 144–145 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 8.81 (1 H, d, J = 8.2 Hz, C-4H), 8.73 (1 H, dd, J = 7.7, 1.5 Hz, C-5′H), 8.48 (1 H, d, J = 8.2 Hz, C-3H), 8.16 (1 H, dd, J = 7.7, 1.5 Hz, C-3′H), 8.01–7.25 (16 H, m, C-4′H, aromatic H), 6.43 (1 H, s, C-6H), 4.03 (3 H, s, CO_2CH_3).

A suspension of the phosphine imide **23** (20 mg, 0.035 mmol) in 1.2 mL of THF and 0.8 mL of H_2O was treated with 1.2 mL of acetic acid, and the solution was stirred at 25 °C for 12 min. Chromatography (SiO_2, 1 × 11 cm, 80–100% EtOAc–hexane gradient elution) and trituration of purified 7-aminoquinoline-5,8-dione with ether to remove residual triphenylphosphine oxide afforded pure 7-aminoquinoline-5,8-dione **5b** (8.5 mg, 10.9 mg theor, 78%, 78–86%) as a red solid: mp >250 °C; ^{1}H NMR (470 MHz, $CDCl_3$) δ 8.93 (1 H, d, J = 8.3 Hz, C-4H), 8.85 (1 H, d, J = 7.8 Hz, C-5′H), 8.55 (1 H, d, J = 8.3 Hz, C-3H), 8.21 (1 H, d, J = 7.8 Hz, C-3′H), 8.04 (1 H, t, J = 7.8, 7.8 Hz, C-4′H), 6.11 (1 H, s, C-6H), 5.34 (2 H, br s, NH_2), 4.05 (3 H, s, CO_2CH_3); IR (KBr) ν_{max} 3420, 3332, 1715, 1694, 1602, 1582, 1377, 1322, 1244, 1133, 830, 752 cm^{-1}; EIMS, m/e (relative intensity) 309 (M^+, 2), 251 (base); CIMS (NH_3), m/e (relative intensity) 310 (M + 1, base); HRMS, m/e for $C_{16}H_{11}N_3O_4$ calcd 309.0749, found 309.0746.

Anal. Calcd. for $C_{16}H_{11}N_3O_4$: C, 62.13; H, 3.59; N, 13.59. Found: C, 62.40; H, 3.75; N, 13.26.

7-Amino-5,8-dioxo-2-(2′-pyridyl)quinoline-6′-carboxylic Acid (5a). A stirred suspension of **5b** (3.7 mg, 0.012 mmol) in 0.5 mL of THF and 0.4 mL of water under a N_2 atmosphere was treated with a solution of sodium dithionite ($Na_2S_2O_4$, 2.2 mg, 0.0126 mmol, 1.05 equiv) in 0.1 mL of H_2O at 25 °C. After 0.5 h, 1 N aqueous potassium hydroxide (0.084 mL, 0.084 mmol, 7 equiv) was added to the reaction mixture and stirring was continued at 25 °C for 1 h. The reaction mixture was diluted with water (10 mL), acidified with the addition of 10% aqueous HCl solution, and extracted with EtOAc (5 × 20 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was washed with hexane to afford pure **5a** (3.2 mg, 3.5 mg theor, 90%) as an orange solid: mp >250 °C; ^{1}H NMR (470 MHz, Me_2SO-d_6) δ 8.88 (1 H, d, J = 8.2 Hz, C-4H), 8.71 (1 H, t, J = 8.1, 8.1 Hz, C-5′H), 8.48 (1 H, t, J = 8.1, 8.1 Hz, C-3′H), 8.24 (1 H, t, J = 8.1, 8.1 Hz, C-4′H), 8.19 (1 H, d, J = 8.2 Hz, C-3H), 6.10 (2 H, br s, NH_2), 5.91 (1 H, s, C-6H); IR (KBr) ν_{max} 3441, 3342, 1704, 1636, 1611, 1585, 1454, 1356, 1260, 1195, 1040, 996, 883, 782 cm^{-1}; CIMS (NH_3), m/e (relative intensity) 298 (M + 2H + 1, base), 296 (M + 1, 42); HRMS, m/e for $C_{15}H_9N_3O_4$ calcd 295.0593, found 295.0584.

Anal. Calcd. for $C_{15}H_9N_3O_4$: C, 61.02; H, 3.07; N, 14.23. Found: C, 61.22; H, 3.22; N, 14.02.

Methyl 8-(Benzyloxy)-7-bromo-2-(2′-aminophenyl)-quinoline-5′-carboxylate (27). A solution of methyl 3-acetyl-4-aminobenzoate (**26**;[65b] 193 mg, 1.0 mmol, 1.0 equiv) in tetrahydrofuran (10 mL) was treated with a 40% methanolic solution of N-benzyltrimethylammonium hydroxide (Triton B,[66] 1.67 g, 4.0 mmol, 4.0 equiv) at 0 °C under N_2. A solution of 2-amino-3-(benzyloxy)-4-bromobenzaldehyde (**14**;[55a] 322 mg, 1.05 mmol, 1.05 equiv) in tetrahydrofuran (1.5 mL) was added to the reaction mixture. The reaction mixture was stirred at 0 °C (1 h) and at 25 °C (5 h), diluted with saturated ammonium chloride (15 mL), and further diluted with water (25 mL) before being extracted with EtOAc, (3 × 15 mL). The organic extracts were washed with

saturated aqueous NaCl (25 mL) and dried (Na_2SO_4), and the solvent was removed in vacuo. Chromatography (SiO_2, 1.5 × 27 cm, 10–25% EtOAc–hexane eluant; gradient elution) afforded pure **27** (376 mg, 463 mg theor, 81%) as a yellow solid: **mp** 157.5–158.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.61 (1 H, d, J = 1.9 Hz, C-6′H), 8.24 (1 H, d, J = 8.9 Hz, C-4H), 8.07 (1 H, d, J = 8.9 Hz, C-3H), 7.86 (1 H, dd, J = 8.5, 1.9 Hz, C-4′H), 7.69 (1 H, d, J = 8.8 Hz, C-6H), 7.48 (1 H, d, J = 8.8 Hz, C-5H), 7.36–7.66 (5 H, m, aromatic H), 7.14 (2 H, br s, NH_2), 6.69 (1 H, d, J = 8.5 Hz, C-3′H), 5.31 (2 H, s, CH_2Ph), 3.90 (3 H, s, CO_2CH_3); IR (KBr) ν_{max} 3411, 1695, 1615, 1547, 1503, 1437, 1367, 1299, 1252, 1207, 1085, 965, 843, 766 cm^{-1}; EIMS, m/e (relative intensity) 462/464 (M^+, 1/1, 7); CIMS (NH_3), m/e (relative intensity) 463/465 (M + 1, 1/1, base); HRMS, m/e for $C_{24}H_{19}BrN_2O_3$ calcd 462.0579, found 462.0571.

Methyl 7-Bromo-8-hydroxy-2-(2′-aminophenyl)-quinoline-5′-carboxylate (28). A solution of methyl 8-(benzyloxy)-7-bromo-2-(2′-aminophenyl)quinoline-5′-carboxylate (**27**; 375 mg, 0.18 mmol) in CH_2Cl_2 (10 mL) saturated with HBr gas was warmed at 60 °C for 10 h in a sealed tube.[82] The reaction mixture was cooled, and saturated aqueous $NaHCO_3$ (15 mL) was added with stirring until the yellow suspension dissolved completely. The mixture was diluted with water (25 mL) and extracted with CH_2Cl_2 (7 × 15 mL). The combined organic extracts were washed with saturated aqueous NaCl (25 mL) and dried (Na_2SO_4), and the solvent was removed in vacuo. Washing the residue with hexane (9 × 10 mL) afforded pure **28** (263 mg, 302 mg theor, 87%) as a beige solid: mp 206–207 °C; ^{1}H NMR ($CDCl_3$) δ 8.31 (1 H, d, J = 1.8 Hz, C-6′H), 8.25 (1 H, d, J = 8.7 Hz, C-4H), 7.91 (1 H, dd, J = 8.6, 1.8 Hz, C-4′H), 7.85 (1 H, d, J = 8.7 Hz, C-3′H), 7.63 (1 H, d, J = 8.8 Hz, C-6H), 7.27 (1 H, d, J = 8.8 Hz, C-5H), 6.79 (1 H, d, J = 8.6 Hz, C-3′H), 5.85 (2 H, br s, NH_2), 3.90 (3 H, s, CO_2CH_2); IR (KBr) ν_{max} 3504, 1719, 1617, 1599, 1507, 1437, 1302, 1251, 1215, 1128, 997, 846, 769 cm^{-1}; EIMS, m/e (relative intensity) 372/374 (M^+, 1/1, 63), 313/315 (1/1, 6); CIMS (NH_3), m/e (relative intensity) 373/375 (M + 1, 1/1, base); HRMS, m/e for $C_{17}H_{13}BrN_2O_3$ calcd 372.0109, found 372.0124.

Methyl 7-Bromo-2-(2′-aminophenyl)-5,8-dioxoquinoline-5′-carboxylate (29). A solution of the bromophenol **28** (30 mg, 0.0804 mmol) in CH_2Cl_2 (8 mL) was added dropwise to a solution of potassium nitrosodisulfonate[66] (Fremy's salt, 108 mg, 0.402 mmol, 5 equiv) and tetra-*n*-butylammonium hydrogen sulfate (27.3 mg, 0.0804 mmol, 1 equiv) in 1.0 M phosphate buffer (KH_2PO_4/Na_2HPO_4 = 1, 8 mL) at 25 °C.[69] The two-phase reaction mixture was stirred vigorously at 25 °C. After 8 h, additional Fremy's salt (108 mg, 0.402 mmol, 5 equiv) was added to the reaction mixture and it was further stirred at 25 °C (12 h). The reaction mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (8 × 15 mL). The combined organic extracts were washed with saturated aqueous NaCl (20 mL) and dried (Na_2SO_4). The removal of the solvent in vacuo afforded 46 mg of crude **29** as a purple, insoluble, unstable solid: ^{1}H NMR (Me_2SO-d_6) δ 8.34–8.50 (3 H, m, C-3H, C-4H, C-6′H), 8.27 (2 H, br s, NH_2), 7.86 (1 H, s, C-6H), 7.77 (1 H, dd, J = 8.8, 1.5 Hz), 6.91 (1 H, d, J = 8.8 Hz), 3.84 (3 H, s, CO_2CH_3).

Methyl 7-Azido-2-(2′-aminophenyl)-5,8-dioxoquinoline-5′-carboxylate (30). A stirred suspension of crude bromoquinone **29** (46 mg) in THF–H_2O (1 mL, 4:1) was treated with sodium azide (5.2 mg, 0.0804 mmol) at 25 °C under a N_2 atmosphere, and the reaction mixture was stirred at 25 °C for 0.5 h. The reaction mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (5 × 15 mL). The combined organic extracts were washed with saturated aqueous NaCl (20 mL) and dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was triturated with hexane (2 × 3 mL) to afford pure **30** (25 mg, 0.0716 mmol, 89% from **28**) as a maroon solid: mp 168–169 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 8.45 (1 H, d, J = 1.8 Hz, C-6′H), 8.35–8.43 (2 H, m, C-3H, C-4H), 8.21 (2 H, br s, NH_2), 7.77 (1 H, dd, J = 8.8, 1.8 Hz, C-4′H), 6.91 (1 H, d, J = 8.8 Hz, C-3′H), 6.59 (1 H, s, C-6H), 3.81 (3 H, s, CO_2CH_3); IR (KBr) ν_{max} 2122, 1696, 1652, 1616, 1582, 1506, 1441, 1332, 1291, 1239, 1121, 1002, 857 cm^{-1}; EIMS, m/e (relative intensity) 349 (M^+, 10), 321 (M – N_2, 11), 290 (M – CO_2CH_3, base).

Methyl 7-Amino-2-(2′-aminophenyl)-5,8-dioxoquinoline-5′-carboxylate (6b). A stirred suspension of the azide **30** (25 mg, 0.0716 mmol) in dry CH_2Cl_2–MeOH (3 mL, 2:1) under a N_2 atmosphere was treated with a solution of triphenylphosphine[63,55a] (18.8 mg, 0.0716 mmol, 1.0 equiv) in 0.5 mL of CH_2Cl_2 with stirring. After 1.5 h at 25 °C, the solvent was removed in vacuo. Chromatography (SiO_2, 1 × 19 cm, 40–60% EtOAc–hexane eluant; gradient elution) afforded the phosphine imide **31** (27 mg, 42 mg theor, 65%) as a light brown solid: mp 178–179 °C; ^{1}H NMR ($CDCl_3$) δ 8.45 (1 H, d, J = 1.9 Hz, C-6′H), 8.38 (1 H, d, J = 8.6 Hz, C-4H), 8.10 (1 H, d, J = 8.6 Hz, C-3H), 7.25–7.93 (16 H, m, aromatic H, C-4′H), 6.99 (2 H, br s, NH_2), 6.69 (1 H, d, J = 8.6 Hz, C-3′H), 6.21 (1 H, d, C-6H), 3.88 (3 H, s, CO_2CH_3); IR (KBr) ν_{max} 1710, 1622, 1595, 1542, 1442, 1326, 1280, 1243, 1114, 1069, 1036, 922, 727, 699 cm^{-1}; CIMS (NH_3), m/e (relative intensity) 584 (M + 1, 14), 279 (base); HRMS, m/e for $C_{35}H_{26}N_3O_4P$ calcd 583.1661, found 583.1666.

A suspension of the phosphine imide **31** (24 mg, 0.0411 mmol) in 1.5 mL of THF and 1 mL of H_2O was treated with 1.5 mL of acetic acid, and the solution was stirred at 25 °C for 12 min. The solvent was removed in vacuo. Trituration of the residue with hexane (12 × 3 mL) and chromatography (SiO_2, 1 × 20.5 cm, 40–100% EtOAc–hexane eluant; gradient elution) afforded pure 7-aminoquinoline-5,8-dione **6b** (13.2 mg, 13.3 mg theor, 99%) as a dark red solid: mp >250 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.42 (1 H, d, J = 1.9 Hz, C-6′H), 8.25–8.34 (2 H, m, C-3H, C-4H), 8.12 (2 H, br s, NH_2), 7.75 (1 H, dd, J = 8.7, 1.9 Hz, C-4′H), 7.34 (2 H, br s, NH_2), 6.88 (1 H, d, J = 8.7 Hz, C-3′H), 5.86 (1 H, s, C-6H), 3.80 (3 H, s, CO_2CH_3); IR (KBr) ν_{max} 3410, 3345, 1700, 1618, 1595, 1545, 1507, 1435, 1356, 1295, 1277, 1245, 1124, 1058, 845, 772 cm^{-1}; EIMS, m/e (relative intensity) 323 (M^+, 8), 264 (M – CO_2CH_3, 2); CIMS (NH_3), m/e (relative intensity) 324 (M + 1, base); HRMS, m/e for $C_{17}H_{13}N_3O_4$ calcd 323.0906, found 323.0913.

Anal. Calcd. for $C_{17}H_{13}N_3O_4$: C, 63.15; H, 4.05; N, 13.00. Found: C, 63.01; H, 4.25; N, 12.80.

7-Amino-2-(2′-aminophenyl)-5,8-dioxoquinoline-5′-carboxylic Acid (6a). A stirred suspension of **6b** (6.0 mg, 0.0186 mmol) in 0.6 mL of THF and 0.4 mL of MeOH under a N_2 atmosphere was treated with a solution of sodium dithionite ($Na_2S_2O_4$, 16.2 mg, 0.0928 mmol, 5.0 equiv) in 0.2 mL of H_2O at 25 °C. After 0.5 h, a solution of lithium hydroxide (7.8 mg, 0.186 mmol, 10 equiv) in 0.25 mL of H_2O was added to the reaction mixture at 25 °C. The reaction mixture was warmed at 50 °C, and stirring was continued for 35 h. The reaction mixture was diluted with water (20 mL), acidified with the addition of 10% aqueous HCl solution, and extracted with EtOAc (6 × 15 mL). The combined organic extracts were washed with saturated NaCl (20 mL) and dried (Na_2SO_4), and the solvent was removed in vacuo. Chromatography (SiO_2, 1 × 11.5 cm, 20% MeOH–EtOAc eluant) afforded pure **6a** (3.8 mg, 5.7 mg theor, 66%) as a dark red solid: mp >250 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.39 (1 H, d, J = 1.6 Hz, C-6′H), 8.20–8.33 (2 H, m, C-3H, C-4H), 7.77 (2 H, br s, NH_2), 7.74 (1 H, dd, J = 8.5, 1.6 Hz, C-4′H), 7.32 (2 H, br s, NH_2), 6.80 (1 H, d, J = 8.5 Hz, C-3′H), 5.85 (1 H, s, C-6H); IR (KBr) ν_{max} 3458, 3363, 1696, 1618, 1462, 1367, 1301, 1250, 1164, 1055, 896, 837, 789, 766, 731, 706 cm^{-1}; EIMS, m/e (relative intensity) 309 (M^+, base), 264 (93); CIMS (NH_3), m/e (relative intensity) 310 (M^+ + 1, 86), 266 (base); HRMS, m/e for $C_{16}H_{11}N_3O_4$ calcd 309.0750, found 309.0743.

Anal. Calcd. for $C_{16}H_{11}N_3O_4$: C, 62.13; H, 3.59; N, 13.59. Found: C, 62.41; H, 3.91; N, 13.27.

In Vitro Antimicrobial Activity. Minimum inhibitory concentrations (MIC, μg/mL) were determined by the agar dilution/streak method following previously established procedures.[74]

In Vitro Cytotoxic Activity. The agents were tested for their toxicity to B16 (mouse melanoma) cells and L-1210 (ATCC 219 mouse lymphocytic leukemia) cells in culture as follows: A population of 10^4 cells in 0.5 mL of Dulbecco-modified Eagle's medium (DMEM) containing 5% bovine serum was added to each well of a 24-place cluster dish (Costar, Cambridge, MA). The cultures were incubated at 37 °C for 48 h under 5% CO_2, 95% humidified air. The test substances, dissolved at 10 mg/mL in 95% ethyl alcohol or dimethyl sulfoxide, were added to fresh culture medium to two times the intended test concentration. To each well of the cluster dish was added 0.5 mL of the appropriate "2×" medium so as to constitute 1 mL of medium per well having the desired concentration of test agent. The cultures were incubated for an additional 48 h, and then the number of cells per well was determined by using either a hemocytometer or a Coulter

particle counter (Coulter Electronics, Inc., Hialeah, FL).

The L-1210 cells do not adhere to the culture dish and can be separated from one another by aspirating the culture through the tip of a pipet. Thus dispersed, the cells were suspended in a total volume of 10 mL with a balanced salts solution (Isoton, Coulter Diagnostics, Hialeah, FL) and counted. The B16 cells do attach to the plastic culture surface. The medium was removed from these cultures, and 0.2 mL of 0.01% crystalline porcine trypsin, 0.1% EDTA in divalent cation-free phosphate-buffered saline was added to each well. After 5 min at 37 °C, the culture plates were chilled on ice and 0.1 mL of phosphate-buffered saline was added to each well. The number of cells in the suspensions was then determined. The number of cells per well was plotted as a function of the concentration of the test agent, and the dose that reduced the cell count to 50% of the untreated controls was determined and is reported as the ID_{50}.

The IC_{50} (μg/mL) values for P388 (mouse leukemia, 9PS) and 9KB (human epidermoid carcinoma of the nasopharynx) cytotoxic cell culture activity were determined by following the established protocols of the National Institutes of Health, National Cancer Institute,[78] at the Purdue Cancer Center Cell Culture Laboratory. The KB cell line, derived from a human epidermoid carcinoma (KB-ADL; oral cavity), was supplied by the NCI contractor. The cell culture screen was performed according to the standard protocol.[78] Samples were dissolved in dimethyl sulfoxide before final dilutions to 100, 10, 0.1, 0.01, 0.001 μg/mL in the growth medium. Samples were run in duplicate. The ID_{50} is the dose of sample that inhibits cell growth to 50% of the untreated control values. The ID_{50} values were obtained by extrapolation from the least-squares fit of the dose-response curve. The PS cell line, derived from a mouse lymphocytic leukemia (P388; spleen and lymph nodes), was supplied by the NCI contractor. The cell culture screen was performed as described above.

Each IC_{50} determination was run in duplicate and was repeated with samples **1a**, **1b**, **5a**, **5b**, and **7–10**. The IC_{50} values (<±10%, unless otherwise noted) are detailed in Table I.

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Supplementary Material Available: A table summarizing the results of the complete series of agents evaluated for in vitro antimicrobial and cytotoxic activity (46 compounds), experimental details for the in vitro CCRF-CEM (human lymphoblastic leukemia) cell culture assay, and experimental protocols for the in vivo 6C3HED lymphosarcoma testing (7 pages). Ordering information is given on any current masthead page.

Notes

The Dopaminergic Moiety of the Ergots: A Controversial Topic Studied with Molecular Mechanics

Håkan Wikström,*† Jenn-Huei Lii,‡ and Norman L. Allinger‡

Organic Chemistry Unit, Department of Pharmacology, University of Göteborg, S-400 33 Göteborg, Sweden, and Department of Chemistry, University of Georgia, Athens, Georgia 30602. *Received November 10, 1986*

Conformational analyses of the side chain of model compounds of the in vivo active dopamine receptor agonist 4-[2-(di-*n*-propylamino)ethyl]indole (DPAI) were performed with molecular mechanics calculations. The results from these calculations, together with the possibility of meta hydroxylation of indoles in vivo, led to the proposal of fitting 6-hydroxy-4-[2-(di-*n*-propylamino)ethyl]indole (6-OH-DPAI), (*S*)-5-hydroxy-*N*,*N*-dialkyl-6,7,8,9-tetrahydro-3*H*-benzo[*e*]indol-8-ylamines and (*S*)-5-hydroxy-2-(dialkylamino)tetralins in a common concept, considering both stereochemistry and hydrogen-bond function in such an overlap. This study emphasizes the importance of considering both conformational analysis and the possibilities of metabolic activation when performing structure–activity studies based on flexible compounds and in vivo data. The answer to the question as to which part of the ergot molecule is responsible for its dopaminergic effect is thus ambiguous. It is possible that the pyrrolylethylamine moiety of the ergots contributes to both in vitro and in vivo effects, and that their 13-OH metabolites contribute, possibly significantly, to their in vivo effects.

The beneficial clinical effects of bromocriptine in the treatment of Parkinson's disease have prompted many attempts to deduce which part of the ergoline structure (1) is responsible for its dopamine (DA) receptor agonist effects.[1-4] The most thorough work has been performed by Kornfeld et al.,[5-8] and these authors conclude that it is the pyrrolylethylamine moiety that is the DA pharmacophore. They also emphasize the importance of stereochemical congruence between different structural classes of DA agonists, and they present data on several pyrrole, pyrazole, and other heterocyclic analogues that support their ideas.[9] Basically, the 5*R* absolute configuration of the ergolines (1) and the 6a*R* absolute configuration of apomorphine (2) superimpose, and hydrogen-bond donors are the pyrrole NH and the 11 phenol OH in the ergolines

† University of Göteborg.
‡ University of Georgia.

(1) Camerman, N.; Camerman, A. *Mol. Pharmacol.* **1981**, *19*, 517.
(2) Nichols, D. E. *J. Theor. Biol.* **1976**, *59*, 167.
(3) Nichols, D. E. *Dopamine Receptors*; Kaiser, C., Kebabian, J. W., Eds.; *ACS Symposium Series, No. 224*; American Chemical Society: Washington, DC, 1983; p 201.
(4) Kocjan, D.; Hodoscek, M.; Hadzi, D. *J. Med. Chem.* **1986**, *29*, 1418.
(5) Bach, N. J.; Kornfeld, E. C.; Jones, N. D.; Chaney, M. D.; Dorman, D. L.; Pascal, J. W.; Clemens, J. A.; Smalstig, E. G. *J. Med. Chem.* **1980**, *23*, 481.
(6) Bach, N. J.; Kornfeld, E. C.; Clemens, J. A.; Smalstig, E. G.; Fredrickson, R. C. A. *J. Med. Chem.* **1980**, *23*, 492.
(7) Bach, N. J.; Kornfeld, E. C.; Clemens, J. A.; Smalstig, E. G. *J. Med. Chem.* **1980**, *23*, 812.

0022-2623/87/1830-1928$01.50/0