110570-28-0; β-CCE, 74214-62-3; Ro 15-1788, 78755-81-4; **1a**, 127791-79-1; **1b**, 127791-80-4; **1c**, 127791-81-5; **1d**, 127791-82-6; **1e**, 127791-83-7; **1f**, 127791-84-8; **1g**, 127791-85-9; **1h**, 127791-86-0; **1i**, 81263-57-2; **2a**, 127791-87-1; **2b**, 127818-96-6; **3**, 39208-08-7; **4a**, 127791-88-2; **4b**, 127791-89-3; **4c**, 127791-90-6; **4d**, 127791-91-7; **4e**, 127791-92-8; **4f**, 127791-93-9; **4g**, 127791-94-0; **4h**, 127791-95-1; **5a**, 127791-96-2; **5b**, 127791-97-3; **5c**, 127791-98-4; **5d**, 127791-99-5; **5e**, 127792-00-1; **5f**, 127792-01-2; **5g**, 127792-02-3; **5h**, 127792-03-4; **6a**, 127792-04-5; **6b**, 127792-05-6; **6c**, 127818-77-3; **6d**, 127792-06-7; **6e**, 127792-07-8; **6f**, 127792-08-9; **6g**, 127792-09-0; **6h**, 127792-10-3; **7**, 127792-11-4; **8**, 127792-12-5; **9**, 127792-13-6; **10**, 127792-14-7; **11a**, 127792-15-8; **11b**, 127792-16-9; **12a**, 127792-17-0; **12b**, 127792-18-1; **13**, 127792-19-2; diazepam, 439-14-5; phenacyl bromide, 70-11-1; aniline hydrobromide, 542-11-0.

Heterosubstituted Anthracene-9,10-dione Analogues. The Synthesis and Antitumor Evaluation of 5,8-Bis[(aminoalkyl)amino]naphtho[2,3-*b*]thiophene-4,9-diones

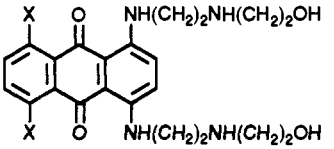
A. Paul Krapcho,* Mary E. Petry, and Miles P. Hacker

Departments of Chemistry and Pharmacology, The University of Vermont, Burlington, Vermont 05405.
Received February 1, 1990

A number of 5,8-bis[(aminoalkyl)amino]naphtho[2,3-*b*]thiophene-4,9-diones have been synthesized and evaluated for antitumor activity against L1210 leukemia both in vitro and in vivo. Two of the congeners exhibited in vivo activities quite comparable to that of mitoxantrone.

The anthracycline antibiotics daunorubicin and doxorubicin have established places in the chemotherapeutic control of cancer.[1] One severe drawback in their use is the risk of severe, dose-related cardiotoxicity.[2] The expression of the cytotoxicity of doxoxrubicin is not well-understood and it would appear that multiple pathways may exist.[3] One rationale which has been utilized for the synthesis of congeners with high antitumor and low cardiotoxic potential is based on the assumption that the cardiotoxicity, at least in part, may be associated with the formation of reactive oxygen species (radical cycling) which attack heart cell membrane lipids. The cardiac tissue, due to its relative lack of endogenous free-radical scavengers, is more susceptible to damage. This has led to the preparation and biological evaluations of chromophore-modified anthracyclines with lower reduction potentials than doxorubicin. Several of the synthetics such as 5-iminodaunorubicin[4] and 5-iminodoxorubicin[5] have been reported to have significantly reduced cardiotoxicies relative to doxorubicin. Xanthone-[6] and thioxanthone-[7] modified anthracyclines have also been studied. Attempts to prepare N^9,N^{10}-dioxides tetrahydrobenzo[*b*]phenazines have been unsuccessful.[8]

The anthracene-9,10-diones ametantrone (**1a**) and mitoxantrone (**1b**) have been shown to have outstanding antitumor activities[9] but a much narrower spectrum of activity in comparison with those of the anthracyclines. Although early studies indicated that mitoxantrone (**1b**) was noncardiotoxic, clinical cardiotoxicity has since been reported.[10]



1a: X = H
b: X = OH

In order to more fully evaluate the structure–activity relationship for ametantrone and mitoxantrone, many 9,10-dione congeners with the sidearms occupying different

(1) (a) Arcamone, F. *Doxorubicin Anticancer Antibiotics: Medicinal Chemistry*; Academic Press: New York, 1981; Vol. 17. (b) Arcamone, F. *Med. Res. Rev.* **1984**, *4*, 153. (c) Arcamone, F. *Cancer Res.* **1985**, *45*, 5995. (d) Crooke, S. T.; Reich, S. D., Eds. *Anthracyclines: Current Status and New Development*; Academic Press: New York, 1980. (e) Beckman, R. A.; McFall, P. J.; Sikic, B. I.; Smith, S. D.; *JNCI, J. Natl. Cancer Inst.* **1988**, *80*, 361. (f) Weiss, R. B.; Sarosy, G.; Clagett-Carr, K.; Russo, M.; Leyland-Jones, B. *Cancer Chemother. Pharmacol.* **1986**, *18*, 185.

(2) Speyer, J. L.; Green, M. D.; Kramer, E. *New Engl. J. Med.* **1988**, *319*, 745.

(3) (a) Fisher, J. F.; Aristoff, P. A. *Prog. Drug Res.* **1988**, *32*, 411. (b) Abdella, B. R. J.; Fisher, *J. Environ. Health Perspect.* **1985**, *64*, 3. (c) *Anthracycline and Anthracenedione-Based Anticancer Agents, Bioactive Molecules*; Lown, J. W., Ed.; Elsevier; Amsterdam, 1988; Vol. 6.

(4) (a) Tong, G. L.; Henry, D. W.; Acton, E. M. *J. Med. Chem.* **1979**, *22*, 36. (b) Lown, J. W.; Chen, H. H.; Plambeck, J. A.; Acton, E. M. *Biochem. Pharm.* **1979**, *28*, 2563. (c) Lown, J. W.; Chen, H. H.; Plambeck, J. A.; Acton, E. M. *Biochem. Pharm.* **1982**, *31*, 574. (d) Myers, C. E.; Muindi, J. R. F.; Zweiter, J.; Sinha, B. K. *J. Biol. Chem.* **1987**, *262*, 11571.

(5) (a) Acton, E. M.; Tong, G. L. *J. Med. Chem.* **1981**, *24*, 669. (b) Bird, D. M.; Boldt, M.; Koch, T. H. *J. Am. Chem. Soc.* **1987**, *109*, 4046.

(6) (a) Lown, J. W.; Sondhi, S. M.; Mandal, S. B.; Murphy, J. *J. Org. Chem.* **1982**, *47*, 4304. (b) Lown, J. W.; Sondhi, S. M. *J. Org. Chem.* **1984**, *49*, 2844. (c) Lown, J. W.; Sondhi, S. M. *J. Org. Chem.* **1985**, *50*, 1413. (d) Charlton, J. L.; Sayeed, V. A.; Lypka, G. N. *Can. J. Chem.* **1982**, *60*, 1996.

(7) Wong, C. M.; Mi, A. Q.; Ren, J.; Lam, H. Y.; Marat, K. *Can. J. Chem.* **1984**, *62*, 1600.

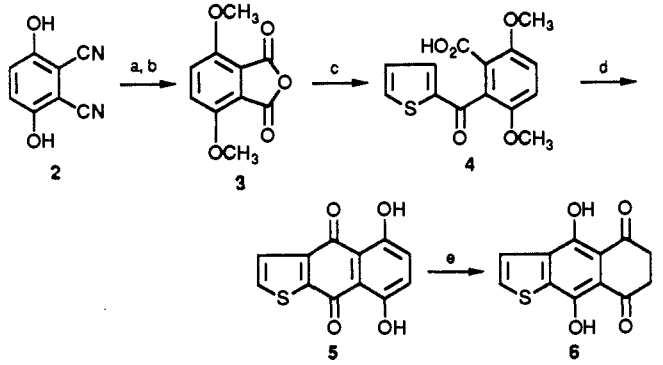
(8) Acton, E. M.; Tong, G. L. *J. Heterocycl. Chem.* **1981**, *18*, 1141.

(9) (a) Murdock, K. C.; Child, R. G.; Fabio, P. F.; Angier, R. B.; Wallace, R. E.; Durr, F. E.; Citarella, R. V. *J. Med. Chem.* **1979**, *22*, 1024. (b) Zee-Cheng, R. K. Y.; Cheng, C. C. *J. Med. Chem.* **1978**, *21*, 291. (c) Wallace, R. E.; Murdock, K. C.; Angier, R. B.; Durr, F. E. *Cancer Res.* **1979**, *39*, 1570. (d) Cheng, C. C.; Zee-Cheng, R. K. Y. in *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier: Amsterdam, 1983; pp 83 and references cited therein. (e) Zee-Cheng, R. K. Y.; Cheng, C. C. *Drugs Future* **1983**, *8*, 229. (f) Shenkenberg, M. D.; Von Hoff, D. D. *Ann. Int. Med.* **1986**, *105*, 67.

(10) (a) Tumminello, F. M.; Leto, G.; Gebbia, N.; Gebbia, V.; Russo, A.; Rausa, L. *Cancer Treat. Rep.* **1987**, *71*, 529. (b) Perkin, W. E.; Schroeder, R. L.; Carrano, R. A.; Imondi, A. R. *Cancer Treat. Rep.* **1984**, *68*, 841. (c) Backmann, E.; Weber, E.; Zbinden, G. *Cancer Treat. Rep.* **1987**, *71*, 361. (d) Tham, P.; Dougherty, W.; Iatropoulos, M. J.; Gordon, G.; James, V. C.; Hall, C.; Noble, J. F. *Am. J. Pathol.* **1987**, *128*, 121.

0022-2623/90/1833-2651$02.50/0

Scheme I[a]

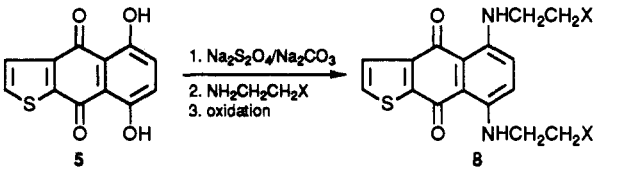


[a] Reagents and conditions: (a) $(CH_3O)_2SO_2$/2-butanone/K_2CO_3/heat, (b) concentrated H_2SO_4/heat, (c) (i) 2-bromothiophene/Mg/Et_2O added to **3** in benzene, (ii) H_2O/acetic acid, (d) concentrated H_2SO_4/150 °C, (e) Zn/acetic acid/heat.

positions on the carbocyclic skeleton have been synthesized and evaluated for antitumor activity. Anthracene-9,10-diones substituted with 1,4-bis[(aminoalkyl)amino],[11] 1,5-bis[(aminoalkyl)amino],[12] 1,8-bis[(aminoalkyl)amino],[12,13] 1,4-bis(aminoalkanamido),[14] and 1,4-bis[(hydroxyalkyl)amino][15] sidearms have been studied. Several chloro- and dichloro-substituted analogues have also been reported.[16] Congeners related to chrysophanol and emodin have been recently evaluated as antitumor agents.[17]

As in the case of the anthracyclines, the mechanisms of cell kill for the anthracene-9,10-diones are poorly understood. Interactions with DNA,[18] free-radical formation (radical cycling),[19] topoisomerase interactions,[20] and/or membrane interactions[21] may play roles in the expressed cytotoxicity.

Table I. 5,8-Bis[(aminoalkyl)amino]naphtho[2,3-*b*]thiophene-9,10-diones



struct 8	X	% yield	struct 8	X	% yield
a	$N(CH_3)_2$	60	d	NH_2[a]	99
b		8	e	OH	76
c	$NHCO_2$-*t*-Bu	25	f	$NH(CH_2)_2OH$	*b*

[a] As the dihydrochloride salt from treatment of **8c** with dry HCl gas. [b] Product detectable by TLC and NMR but difficult to isolate.

Partially on the basis of the redox-cycling supposition previously utilized for anthracycline analogue design, chromophore-modified anthracene-9,10-diones congeners with less tendency to be reduced to semiquinone free radicals than mitoxantrone have been studied. Analogues of anthrapyrazoles,[22] benzothiopyranoindazoles,[23] 1-aminoxanthones,[24] and 1-aminothioxanthones[25] have been synthesized and several of these have been extensively evaluated for their antitumor properties.

Our research in this area has focused on the synthesis of heterosubstituted analogues related to the anthracene-9,10-diones. We have recently reported on the antitumor evaluations of 5,8-bis[(aminoalkyl)amino]-1-azaanthracene-9,10-diones.[26] Other aza analogues are currently being evaluated for antitumor activity.[27]

The goals of this investigation are the synthesis and biological evaluation of thiophene-annulated naphtho-

(11) (a) Stefanska, B.; Dzieduszycka, M.; Martelli, S.; Borowski, E. *Eur. J. Med. Chem.* **1989**, *32*, 1725. (b) Krapcho, A. P.; Landi, J. J., Jr.; Phinney, D. G.; Hacker, M. P.; McCormack, J. J. *J. Med. Chem.* **1986**, *29*, 1370. (c) Krapcho, A. P.; Shaw, K. J.; Landi, J. J., Jr.; Phinney, D. G. *J. Org. Chem.* **1984**, *49*, 5253. (d) Krapcho, A. P.; Getahun, Z. *Syn. Commun.* **1985**, *15*, 907. (e) Dzieduszycka, M.; Stefanska, B.; Kolodzieiczyk, P.; Borowski, E. *Farmaco, Ed. Sci.* **1987**, *42*, 219. (f) Mathew, E. E.; Cheng, C. C. *Tetrahedron Lett.* **1984**, *25*, 269.

(12) Katzhendler, J.; Gean, K.-F.; Bar-Ad, G.; Tashma, Z.; Ben-Shoshan, R.; Ringel, I.; Bachrach, U.; Ramu, A. *Eur. J. Med. Chem.* **1989**, *24*, 23.

(13) Krapcho, A. P.; Getahun, Z.; Hacker, M. P.; McCormack, J. J. *Bull. Chem. Soc. Ethiop.* **1988**, *2*, 69.

(14) (a) Martelli, S.; Dzieduszycka, M.; Stefanska, B.; Bontemps-Gracz, M.; Borowski, E. *J. Med. Chem.* **1988**, *31*, 1956. (b) Antonello, C.; Uriarte, E.; Palumbo, M. *Arch. Pharm. (Weinheim, Ger.)* **1988**, *321*, 513. (c) Collier, D. A.; Neidle, S. *J. Med. Chem.* **1988**, *31*, 847.

(15) (a) Andreani, A.; Rambaldi, M.; Bonazzi, D.; Lelli, G.; Greci, R.; Ross, R.; Galatulas, I. *Arch. Pharm. (Weinheim, Ger.)* **1985**, *318*, 400. (b) Andreani, A.; Rambaldi, M.; Bonazzi, D.; Lelli, G.; Bossa, R.; Galatulas, I. *Arch. Pharm. (Weinheim, Ger.)* **1985**, *318*, 842.

(16) Zee-Cheng, R. K.-Y.; Mather, A. E.; Xu, P.; Northcutt, R. V.; Cheng, C. C. *J. Med. Chem.* **1987**, *30*, 1682.

(17) Koyama, M.; Takahashi, K.; Chou, T.-C.; Darzynkiewicz, Z.; Kapuscinski, J.; Kelley, T. R.; Watanabe, K. A. *J. Med. Chem.* **1989**, *32*, 1594.

(18) (a) Sinha, B. K. *Chem. Biol. Interact.* **1989**, *69*, 293. (b) Hurley, L. H. *J. Med. Chem.* **1989**, *32*, 2027. (c) Bowden, G. T.; Roberts, R.; Alberts, D. S.; Peng, Y. M.; Garcia, D. *Cancer Res.* **1985**, *45*, 4915. (d) Kapuscinski, J.; Darzynkiewicz, Z. *Proc. Natl. Acad. Sci.* **1986**, *83*, 6302.

(19) Basra, J.; Wolf, C. R.; Brown, J. R.; Patterson, L. H. *Anti-Cancer Drug Des.* **1985**, *1*, 45.

(20) (a) Rose, K. M. *Fed. Am. Soc. Exp. Biol. J.* **1988**, *2*, 2474. (b) Wang, J. C. *Biochem. Biophys. Acta* **1987**, *909*, 1. (c) Crespi, M. D.; Invanier, S. E.; Genovese, J.; Baldi, A. *Biochem. Biophys. Res. Commun.* **1986**, *136*, 521.

(21) Burke, T. G.; Sartorelli, A. C.; Tritton, T. R. *Cancer Chemother. Pharmacol.* **1988**, *21*, 274.

(22) (a) Showalter, H. D. H.; Johnson, J. L.; Werbel, L. M.; Leopold, W. R.; Jackson, R. C.; Elslager, E. F. *J. Med. Chem.* **1984**, *27*, 255. (b) Showalter, H. D. H.; Johnson, J. L.; Hoftiezer, J. M.; Turner, W. R.; Werbel, L. M.; Leopold, W. R.; Shillis, J. L.; Jackson, R. C.; Elslager, E. F. *J. Med. Chem.* **1987**, *30*, 121. (c) Showalter, H. D. H.; Johnson, J. L.; Hoftiezer, J. M.; *J. Heterocycl. Chem.* **1986**, *23*, 1491. (d) Leopold, W. R.; Nelson, J. M.; Plowman, J.; Jackson, R. C. *Cancer Res.* **1985**, *45*, 5532. (e) Showalter, H. D. H.; Fry, D. W.; Leopold, W. R.; Lown, J. W.; Plambeck, J. A.; Reszka, K. *Anti-Cancer Drug Des.* **1986**, *1*, 73. (f) Beylin, V. G.; Colbry, N. L.; Goel, O. P.; Haky, J. E.; Johnson, D. R.; Johnson, J. L.; Kanter, G. D.; Leeds, R. L.; Leja, B.; Lewis, E. P.; Rithner, C. D.; Showalter, H. D. H.; Sercel, A. D.; Turner, W. R.; Uhlendorf, S. E. *J. Heterocycl. Chem.* **1989**, *26*, 85. (g) Fry, D. W.; Boritzki, T. J.; Besserer, J. A.; Jackson, R. C. *Biochem. Pharmacol.* **1985**, *34*, 3499. (h) Sebolt, J.; Havlick, M.; Hamelehle, K.; Nelson, J.; Leopold, W.; Jackson, R. *Cancer Chemother. Pharmacol.* **1989**, *24*, 219. (i) Frank, S. K.; Mathiesen, D. A.; Szurszewski, M.; Kuffel, M. J.; Ames, M. M. *Cancer Chemother. Pharmacol.* **1989**, *23*, 213.

(23) (a) Showalter, H. D. H.; Angelo, M. M.; Berman, E. M.; Kanter, G. D.; Ortwine, D. F.; Ross-Kesten, S. G.; Sercel, A. D.; Turner, W. R.; Werbel, L. M.; Worth, D. F.; Elslager, E. F.; Leopold, W. R.; Shillis, J. L. *J. Med. Chem.* **1988**, *31*, 1527. (b) Fry, D. W.; Besserer, J. A. *Mol. Pharmacol.* **1988**, *33*, 84.

(24) Mancini, M. L.; Honek, J. F. *Syn. Commun.* **1989**, *19*, 2001.

(25) (a) Mancini, M. L.; Honek, J. F. *Syn. Commun.* **1986**, *16*, 1109. (b) Archer, S.; Miller, K. J.; Rej, R.; Periana, C.; Fricker, L. *J. Med. Chem.* **1982**, *25*, 220. (c) Archer, S.; Rej, R. *J. Med. Chem.* **1982**, *25*, 328.

(26) Krapcho, A. P.; Landi, J. J., Jr.; Hacker, M. P.; McCormack, J. J. *J. Med. Chem.* **1985**, *28*, 1124.

(27) Petry, M. E., Ph.D. thesis, University of Vermont, May 1989.

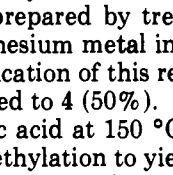
quinones bearing (aminoalkyl)amino side arms at positions identical with those found in ametantrone (**1a**) or mitoxantrone (**1b**). These thiophene analogues are chromophore-modified anthracene-9,10-diones with significantly different electronic characteristics and would be expected to undergo electron uptake with more difficulty in comparison with the carbocyclic anthracene-9,10-diones. In addition, these chemotypes should maintain the planarity and spatial and electronic characteristics of the carbocyclic quinone model which may be necessary for molecular recognition at the cellular level (e.g. intercalation).

Chemistry

The target molecule for the synthesis of the desired 5,8-bis[(aminoalkyl)amino]naphtho[2,3-*b*]thiophene-4,9-diones was 6,7-dihydro-4,9-dihydroxynaphtho[2,3-*b*]-thiophene-5,8-dione (**6**). The methodology for its preparation is outlined in Scheme I.

Commercially available 2,3-dicyanohydroquinone (**2**) was treated with dimethyl sulfate to yield the corresponding dimethoxyhydroquinone in a quantitative yield. Hydrolysis of this dicyano compound with concentrated sulfuric acid gave anhydride **3** (84%). The Grignard reagent which was prepared by treatment of 2-bromothiophene with magnesium metal in ether was added to **3** in benzene. Acidification of this reaction mixture with aqueous acetic acid led to **4** (50%). Treatment of **4** with concentrated sulfuric acid at 150 °C for 3 h resulted in cyclization and demethylation to yield **5** (66%). The reduction of **5** to **6** (85%) was effected by zinc and acetic acid.

The structural assignment for the reduction product as **6** is based on ^{1}H and ^{13}C NMR comparisons with those reported for leucoquinizarin (**7**).[28] Compound **6** has ab-



7

sorptions for the methylene protons at δ 3.10 ($CDCl_3$) and the methylene carbons at 36.2 ppm ($CDCl_3$, only one methylene peak was detected). The carbonyl carbon was found at 201.3 ppm (only one absorption was detected). Leucoquinizarin (**7**) exhibits its methylene absorptions at 3.05 ppm and in the ^{13}C NMR has the methylene peaks at 35.7 and the carbonyl carbons at 200.8 ppm.

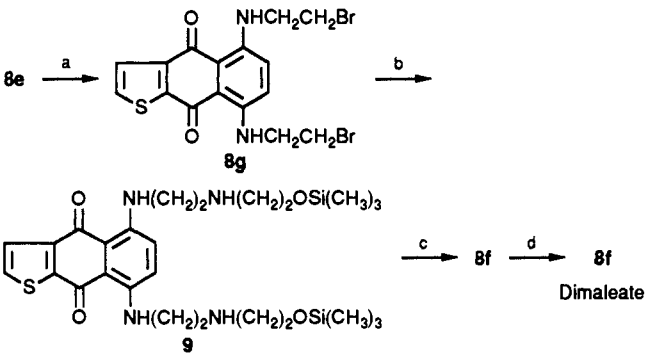
The amine condensations with **6** were performed in two ways. The leuco compound **6** was treated with the amine to yield the corresponding bis-imine which on air oxidization led to the desired compound. The more expedient way was to generate **6** (in situ preparation by treatment of **5** with sodium dithionite) followed by addition of the appropriate amine and air oxidation. In general, this reaction sequence led to the desired products in reasonable yields. The results are tabulated in Table I.

When *N*-(2-aminoethyl)ethyleneimine was used as the amine a particularly low yield of **8b** was obtained. This may be attributed to reaction of the amine side chain with the excess amine present in the reaction mixture. A significant amount of a tarry blue mass remained which was insoluble in a range of solvents.

Treatment of **6** with *N*-(*tert*-butoxycarbonyl)ethylenediamine (a synthon for ethylenediamine) led to **8c**. The

(28) (a) Kikuchi, M.; Yamagishi, T.; Hida, M. *Dyes Pigm.* **1981**, *2*, 143. (b) Hofle, G. *Tetrahedron* **1977**, *33*, 1963. (c) Brand, D. J.; Fisher, J. *J. Am. Chem. Soc.* **1986**, *108*, 3088.

Scheme II[a]



[a] Reagents and conditions: (a) $(C_6H_5)_3P/CBr_4$, (b) $H_2N(CH_2)OSi(CH_3)_3$, (c) silica gel chromatography, (d) maleic acid.

electron-withdrawing effect of the *t*-BOC group on the terminal nitrogen prevented the formation of undesirable tetrahydroquinoxaoline. The *t*-BOC group was readily removed by treatment of a chloroform solution of **8c** with hydrogen chloride gas to yield **8d**.

Attempts to prepare ametantrone analogue **8f** by condensation of **6** with 2-[(2-aminoethyl)amino]ethanol were met with difficulty. Analysis of the crude reaction mixture by TLC showed a very polar blue spot indicative of the desired **8f** (based also on ^{1}H NMR analysis of the crude product). Unfortunately, the polarity of the product and the nature of the amine itself caused extreme difficulty in separation of the two components. Separation by chromatography or crystallization were ineffective and led to tetrahydroquinoxaline formation.

Because of the difficulties of isolating **8f** directly from condensations involving the leuco compound, an alternative preparative route which is depicted in Scheme II was used.

Treatment of **8e** with triphenylphosphine and carbon tetrabromide gave dibromo derivative **8g** (48%). Initially, the bromides were displaced by 2-aminoethanol; however, difficulty was encountered in the complete removal of the excess amine. To circumvent this problem, the 2-aminoethanol was O-silylated to produce a more volatile amino derivative. Displacements of the bromides by 2-(trimethylsiloxy)ethylamine gave O-silyl derivative **9**. On purification of this compound by column chromatography on silica gel, the O–Si bond was cleaved to give **8f** (28%). This compound was converted to the maleate salt by treatment with maleic acid. A second route to **8f** (30%) involved acid-catalyzed aziridine ring-opening of **7b**. However, the difficulty involved in the preparation of the aziridine compound made this a less viable synthetic route.

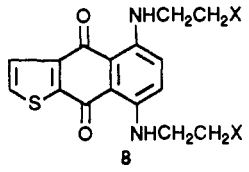
Biological Data

All of the 5,8-bis(amino-substituted)naphtho[2,3-*b*]-thiophene-4,9-diones were biologically evaluated in vitro and the active compounds were further evaluated in an in vivo L1210 mouse tumor screen. These results are tabulated in Table II.

As in the carbocyclic series,[9] analogues **8e** and **8g**, which lack amino sidearm substitution, showed little in vitro activity as anticipated. The dimethylamino analogue **8a** which exhibited high in vitro activity was found to be inactive in the L1210 and P388 screens. The aziridine derivative **8b** was quite potent in the in vitro study but did not manifest this activity in the in vivo screen.

On the other hand, the amino analogues **8d** and **8f** demonstrate impressive activities both in vitro and in vivo. In order to have a direct cytotoxicity comparison model, ametantrone (**1a**) was evaluated in our L1210 in vivo

Table II. Cytotoxic and Antitumor Activities of the 5,8-Bis[(aminoalkyl)amino]naphtho[2,3-*b*]thiophene-4,9-diones against L1210 Leukemia



8

8, X	ID_{50}, μg/mL	dose, mg/kg[a]	% T/C	60-day survivors
a, $N(CH_3)_2$	0.038	50 × 3	109	0/6
		25 × 3	107	0/6
a, $N(CH_3)_2$[b]		100 × 1	toxic	
		50 × 2	128	0/6
		25 × 2	108	0/6
	0.0035	50 × 2	114	0/6
		25 × 2	112	0/6
		12.5 × 2	104	0/6
d, NH_2[c]	0.026	12.5 × 2	386	1/6
		8 × 3	400	1/6
		4 × 3	325	3/6
e, OH	>10			
f, $NHCH_2CH_2OH$[d]	0.064	25 × 3	333	3/6
		6.25 × 3	403	1/6
g, Br	5.2			

[a] Dose schedule: ×2 (days 1 and 5); ×3 (days 1, 5, and 9). [b] P388. [c] As the dihydrochloride salt. [d] As the dimaleate salt.

screen. At doses of 50, 25, and 12.5 mg/kg (days 1, 5 and 9) **1a** gave % T/C values of 146, 203 (1/6 60-day survivors) and 177, respectively. Analogues **8d** and **8f** are considerably more active than ametantrone. In fact the antitumor activities of **8d** and **8f** are quite comparable with that of mitoxantrone.[9c]

Further pharmacological and mechanistic studies of **8d** and **8f** are in progress.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Proton and ^{13}C NMR were run on a Bruker WP-270SY or WM-250 pulsed Fourier transform spectrometer. Precoated silica gel and alumina plates (Eastman chromagram sheets) with fluorescent indicator were used for thin-layer chromatography. Baker analyzed 80–200 mesh silica gel was used for column chromatography. Mass spectra were run on a Finnigan MAT 4610 spectrometer. Microanalyses were performed by Robertson Laboratory, Madison, NJ.

Synthetic Procedures. 1,4-Dimethoxy-2,3-dicyanobenzene. Dimethyl sulfate (15 mL, 0.16 mol) and anhydrous potassium carbonate (24 g, 0.17 mol) were added to a solution of 2,3-dicyanohydroquinone (3.0 g, 0.019 mol) in 2-butanone (100 mL). The reaction mixture was refluxed for 18 h, cooled to room temperature and the solid was collected by filtration. The residue was added to water (100 mL) to dissolve the potassium carbonate and the insoluble material was collected by filtration. Recrystallization from acetic acid yielded white needles (3.17 g, 88%): mp 273–274 °C (lit.[29] mp 275 °C); ^1H NMR (DMSO-d_6) δ 7.63 (s, 2 H), 3.93 (s, 6 H).

3,6-Dimethoxyphthalic Anhydride (3). A mixture of 1,4-dimethoxy-2,3-dicyanobenzene (3.14 g, 0.017 mol) in concentrated sulfuric acid (15 mL) was heated on a steam bath for 1 h. After standing at room temperature overnight, the solid was collected by filtration, washed with water, and dried to yield a yellowish powder (2.90 g, 84%): mp 256–258 °C (lit.[29] mp 259–260 °C); ^1H NMR (DMSO-d_6) δ 7.61 (s, 2 H), 3.94 (s, 6 H).

2-(2-Thenoyl)-3,6-dimethoxybenzoic Acid (4). The Grignard reagent was prepared by dropwise addition of a solution of 2-bromothiophene (1.68 g, 0.010 mol) in dry ether (5 mL) into a mixture of magnesium (0.34 g, 0.014 mol) in dry ether (15 mL). Some heat was required to initiate the reaction. The mixture was refluxed for 1 h and then cooled to 20 °C.

A suspension of **3** (2.5 g, 0.012 mol) in dry benzene (50 mL) was warmed to 40 °C and the Grignard reagent was added slowly. The mixture was refluxed for 1.5 h. Upon cooling to room temperature, cold water (40 mL) and magnesium oxide (0.5 g) were added, and the mixture was stirred overnight. The mixture was filtered and the aqueous layer was removed and acidified with acetic acid. The product was collected by filtration and recrystallized from a 1:1 mixture of toluene/ethanol to yield a beige solid (1.3 g, 50%): mp 224–226 °C; ^1H NMR (DMSO-d_6) δ 12.98 (s, 1 H), 8.02 (d, 1 H), 7.37 (d, 1 H), 7.21 (s, 2 H), 7.18 (m, 1 H), 3.81 (s, 3 H), 3.67 (s, 3 H). Anal. ($C_{14}H_{12}O_5S$) C, H.

5,8-Dihydroxynaphtho[2,3-*b*]thiophene-4,9-dione (5). A solution of **4** (0.1 g, 0.34 mmol) in concentrated sulfuric acid (2 mL) was heated in an oil bath maintained at 150 °C for 3 h. The reaction mixture was poured over ice and the product was extracted with chloroform. Recrystallization of this crude material from toluene led to a dark red solid (55 mg, 66%): mp 207–208 °C; ^1H NMR (DMSO-d_6) δ 12.53 (s, 1 H), 12.30 (s, 1 H), 8.15 (d, 1 H), 7.59 (d, 1 H), 7.25 (s, 2 H). Anal. ($C_{12}H_6O_4S$) C, H.

6,7-Dihydro-4,9-dihydroxynaphtho[2,3-*b*]thiophene-5,8-dione (6). A solution of **5** (0.10 g, 0.41 mmol) in glacial acetic acid (7 mL) was brought to a gentle reflux under a nitrogen blanket. Zinc (0.12 g, 1.8 mmol) was added to the mixture, which was then refluxed for 0.5 h. The solution was filtered while hot to remove residual zinc. After cooling, the filtrate was neutralized with 10% sodium hydroxide. The product was collected by filtration, washed well with water, and dried to yield a yellowish-brown powder (85 mg, 85%): mp 177–179 °C; ^1H NMR ($CDCl_3$) δ 13.09 (s, 1 H), 13.02 (s, 1 H), 7.76 (d, 1 H), 7.69 (d, 1 H), 3.10 (s, 4 H); ^{13}C NMR ($CDCl_3$) δ 201.3, 152.9, 152.2, 137.3, 136.9, 131.8, 123.2, 109.7, 108.6, 36.2. Anal. ($C_{12}H_8O_4S$) C, H.

5,8-Bis[[2-(dimethylamino)ethyl]amino]naphtho[2,3-*b*]thiophene-4,9-dione (8a). *N,N*-Dimethylethylenediamine (1.5 mL) was stirred under a nitrogen blanket for 0.25 h and **6** (85 mg, 0.34 mmol) was added. The reaction mixture was stirred under nitrogen at 50–60 °C for 24 h. After cooling to room temperature, the mixture was stirred in air for several hours and then diluted with water. The product was extracted with chloroform; the extract was concentrated and added to a silica gel column. A major blue band was eluted with 5% triethylamine in chloroform. The product was recrystallized from a mixture of chloroform and ligroin to yield dark blue needles (31 mg, 23%): mp 160–162 °C; ^1H NMR ($CDCl_3$) δ 10.93 (s, 1 H), 10.82 (s, 1 H), 7.68 (d, 1 H), 7.55 (d, 1 H), 7.26 (s, 2 H), 3.51 (q, 4 H), 2.67 (t, 4 H), 2.37 (s, 12 H); UV absorption max, nm (2-methoxyethanol) 570 (sh, 6900), 610 (14 700), 659 (18 500). Anal. ($C_{20}H_{26}N_4O_2S$) C, H, N.

5,8-Bis[[2-(1-aziridino)ethyl]amino]naphtho[2,3-*b*]thiophene-4,9-dione (8b). A solution of *N*-(2-aminoethyl)-ethyleneimine (700 mg, 8.14 mmol) in pyridine (7 mL) was stirred under a nitrogen blanket for several minutes and **6** (776 mg, 3.13 mmol) was added. The reaction mixture was stirred at room temperature for 48 h and finally in the air for 3 h. After dilution with chloroform, the chloroform extract was washed several times with brine. The organic layer was concentrated and the product was isolated by column chromatography on silica gel by eluting with 5% methanol in chloroform. Recrystallization from a mixture of chloroform and ligroin led to dark blue crystals (93 mg, 8%): mp 134–136 °C; ^1H NMR δ 11.11 (s, 1 H), 11.01 (s, 1 H), 7.68 (d, 1 H), 7.54 (d, 1 H), 7.34 (d, 2 H), 3.66 (q, 4 H), 2.58 (t, 4 H), 1.82 (m, 4 H), 1.25 (m, 4 H); MS *m/z* (relative intensity) 382.2 (69.2, M^+), 283.0 (100), 55.9 (70.0).

5,8-Bis[[2-[*N*-(*tert*-butoxycarbonyl)amino]ethyl]amino]naphtho[2,3-[*b*]thiophene-4,9-dione (8c). A mixture of **5** (0.24 g, 0.97 mmol), sodium carbonate (0.06 g, 0.57 mmol), and sodium dithionite (0.17 g, 0.98 mmol) in dry ethanol (25 mL) was refluxed for 1 h. The *N*-(*tert*-butoxycarbonyl)ethylenediamine (1.4 g, 9.36 mmol) was added and reflux was continued for 18 h. The solvent was removed by evaporation, and water (20 mL) was added to the residue. A blue solid was collected by filtration and was dried. Chromatography over silica gel with elution by a 5% methanol in chloroform mixture gave a major blue band. Recrystallization from a methylene chloride/ligroin mixture gave fine, blue needles (0.130 g, 25%): mp 196–199 °C; ^1H NMR ($CDCl_3$) δ 10.96 (br, 1 H), 10.84 (br, 1 H), 7.62 (d, 1 H), 7.54 (d,

(29) Graves, G. D.; Adams, R. *J. Am. Chem. Soc.* **1923**, *45*, 2446.

1 H), 7.24 (s, 2 H), 5.04 (br, 2 H), 3.55 (m, 4 H), 3.43 (m, 4 H), 1.46 (s, 18 H). Anal. ($C_{26}H_{34}N_4O_6S$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)ethylenediamine.**[30] A solution of BOCON (Aldrich Chemical Co.) in chloroform (10 mL) was added dropwise from an addition funnel to a stirred solution of ethylenediamine (7.2 g, 0.12 mmol) and triethylamine (4.2 mL, 0.03 mmol) in chloroform (20 mL) at room temperature. The mixture was stirred for an additional 4 h. The solvent was evaporated and the residue was dried under vacuum for 18 h. The resulting yellow oil was chromatographed on a silica gel column. The very polar product was eluted with 1:5:5 $Et_3N/CH_3OH/CHCl_3$. TLC analysis showed a single ninhydrin-positive spot. Evaporation of the eluents gave a pale yellow oil (2.10 g, 66%): ^1H NMR ($CDCl_3$) δ 4.93 (br s, 1 H), 3.17 (q, 2 H), 2.80 (t, 2 H), 1.43 (s, 9 H); MS *m/z* (relative intensity) 160 (100, M^+), 105 (40.9).

5,8-Bis[(2-aminoethyl)amino]naphtho[2,3-*b*]thiophene-4,9-dione Dihydrochloride (8d). Hydrogen chloride gas was bubbled through a solution of **8c** (0.12 g, 0.23 mmol) in dry chloroform (6 mL) for 0.5 h. A dark blue solid was collected by filtration (0.091 g, 99%): mp 237–238 °C; ^1H NMR (DMSO-d_6) δ 10.83 (br, 1 H), 10.71 (br, 1 H), 8.16 (br, 6 H), 8.02 (d, 2 H), 7.61 (s, 2 H), 3.78 (m, 4 H), 3.06 (m, 4 H). Anal. ($C_{16}H_{18}N_4O_2S\cdot 2HCl$) C, H, N.

5,8-Bis[(2-hydroxyethyl)amino]naphtho[2,3-*b*]thiophene-4,9-dione (8e). A mixture of **5** (0.263 g, 1.07 mmol), sodium carbonate (0.053 g, 0.50 mmol), and sodium dithionite (0.158 g, 0.91 mmol) in dry ethanol (20 mL) was refluxed for 1 h. The 2-aminoethanol (4.25 g, 70.0 mmol) was added portionwise over 0.25 h and the reflux was continued for 18 h. The ethanol was removed by evaporation and the residue was quenched with water (40 mL). The product was collected by filtration, dried, and recrystallized from methanol to give a dark blue solid (0.270 g, 76%): mp 196–198 °C; ^1H NMR (DMSO-d_6) δ 11.17 (s, 1 H), 11.01 (s, 1 H), 7.94 (d, 1 H), 7.59 (d, 1 H), 7.50 (s, 2 H), 4.98 (t, 2 H), 3.67 (m, 4 H), 3.53 (m, 4 H); MS *m/z* (relative intensity) 332.2 (50.0, M^+), 301.2 (100). Anal. ($C_{16}H_{16}N_2O_4S$) C, H, N.

5,8-Bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-naphtho[2,3-*b*]thiophene-4,9-dione Dimaleate (8f). (a) Via Bromide Displacements. A solution of **8g** (0.24 g, 0.52 mmol) and 2-(trimethylsiloxy)ethylamine[31] (1.40 g, 0.52 mmol) in dry pyridine (3.0 mL) was stirred at room temperature under a nitrogen blanket for 26 h. The pyridine was evaporated, the residue was dissolved in methylene chloride, washed with a saturated sodium bicarbonate solution, and dried over sodium sulfate. Evaporation left an oily, bluish residue which was dried under vacuum overnight. Chromatography on a silica gel column resulted in the cleavage of the Si–O bond. The product was eluted with 1:5:5 $Et_3N/CH_3OH/CHCl_3$. Evaporation left an oily, blue substance (0.068 g, 28%): ^1H NMR 10.98 (br, 1 H), 10.84 (br, 1 H), 7.47 (d, 1 H), 7.39 (d, 1 H), 6.71 (s, 2 H), 3.72 (m, 4 H), 2.94 (m, 4 H), 2.83 (m, 4 H), 2.76 (m, 4 H); UV absorption max, nm (2-methoxyethanol) 570 (sh, 6100), 610 (11 500), 658 (14 100).

Maleate Salt. The free amine was dissolved in a minimal amount of 50% methanol in chloroform and a solution of maleic acid (0.042 g, 0.26 mmol) in methanol (1 mL) was added. The product crystallized on addition of ether and was collected by filtration (0.085 g, 25%): mp 123–125 °C; ^1H NMR (DMSO-d_6) δ 10.83 (br, 1 H), 10.69 (br, 1 H), 8.60 (br, 4 H), 8.06 (d, 1 H) 7.63 (d, 1 H), 7.57 (s, 2 H), 6.07 (s, 4 H), 5.35 (br, 2 H), 3.88 (m, 4 H), 3.74 (m, 4 H), 3.30 (m, 4 H), 3.12 (m, 4 H). Anal. ($C_{20}H_{26}N_4O_4S\cdot 2C_4H_4O_4$) C, H, N.

(30) Essien, H.; Lai, J. Y.; Hwang, K. J. *J. Med. Chem.* **1988**, *31*, 898.

(31) Lukevits, E. Y.; Libert, L. I.; Voronkov, M. G. *J. Gen. Chem. USSR (Engl. Transl.)* **1969**, *39*, 768.

(b) Via Aziridine Ring Opening. Trifluoroacetic acid (0.25 mL) was added to a solution of **8b** (93 mg, 0.24 mmol) in DMSO (2 mL). The mixture was stirred at room temperature for 24 h and the solvent was removed under reduced pressure. The residue was stirred for several hours with saturated sodium bicarbonate (2 mL). The product was isolated by extraction with chloroform. Removal of the chloroform and trituration of the residue with ethanol gave the free base as a dark blue solid (31 mg, 30%), mp 147–149 °C. The ^1H NMR was identical with that of the oil obtained above.

5,8-Bis[(2-bromoethyl)amino]naphtho[2,3-*b*]thiophene-4,9-dione (8g). Triphenylphosphine (0.31 g, 1.17 mmol) was added portionwise to a mixture of **8e** (0.150 g, 0.45 mmol) and carbon tetrabromide (0.39 g, 1.17 mmol) in dry methylene chloride (4 mL). The mixture was stirred at room temperature for 1.5 h. The solvent was removed by evaporation and the residue was chromatographed on a silica gel column eluting with chloroform. Recrystallization from a mixture of chloroform and ligroin gave fine, blue needles (0.100 g, 48%): mp 177–179 °C; ^1H NMR ($CDCl_3$) 10.94 (br s, 1 H), 10.82 (br s, 1 H), 7.68 (d, 1 H), 7.59 (d, 1 H), 7.17 (s, 2 H), 3.83 (m, 4 H), 3.55 (m, 4 H). Anal. ($C_{16}H_{14}Br_2N_2O_2S$) C, H, N.

Biological Studies. In Vitro Cytotoxicity Evaluations. L1210 murine leukemia cells are routinely maintained as suspension cultures in McCoy's 5A medium supplemented with 10% horse serum, glutamine, penicillin, and streptomycin and grown in a humidified environment of 10% carbon dioxide and 90% air at 37 °C. To assess the in vitro toxicity, each compound was dissolved in dimethyl sulfoxide and added to 1 mL of L1210 cells (5×10^4 cells/mL) to attain final concentrations of 0.01, 0.1, and 10 μg of drug/mL of culture. After 72 h of continuous exposure to the drug, the cell concentration was determined with a Coulter counter. Growth inhibition was calculated for each drug concentration by using the following formula:

$$\% \text{ growth inhibition} = \left[1 - \left(\frac{\text{cell number treated}}{\text{cell number DMSO alone}}\right)\right] \times 100$$

The growth inhibition data were then used to calculate the ID_{50} values (the drug concentration required to inhibit cell growth by 50% of control).

In Vivo Efficacy Studies. L1210 murine leukemia cells were maintained in vivo by weekly intraperitoneal (ip) injections of 10^6 cells in BDF_1 mice. For test purposes, mice were inoculated ip with 10^6 L1210 cells and treatment was initiated 24 h later. The desired dose of drug was administered on days 1 and 5 or days 1, 5, and 9. Mice were observed daily for signs of toxicity and survival. The day of death was recorded for all animals that died or were sacrificed during the 60 day study group. The mean survival time (MST) for each treatment group was calculated and the percent T/C was determined using the following formula:

$$\% \text{ T/C} = [(\text{MST treated})/(\text{MST control})] \times 100$$

Acknowledgment. We wish to thank Boehringer Mannheim Italia (Monza, Italy) for partial financial support of this project. We are indebted to Linda Mathews for excellent technical assistance.

Registry No. 1, 40904-87-8; **2**, 4733-50-0; **3**, 14597-12-7; **4**, 128388-15-8; **5**, 76902-58-4; **6**, 128412-16-8; **8a**, 128388-17-0; **8b**, 128388-18-1; **8c**, 128388-19-2; **8d**, 128388-20-5; **8d**·2HCl, 128388-25-0; **8e**, 128388-21-6; **8f**, 128388-22-7; **8f** dimaleate, 128388-23-8; **8g**, 128388-24-9; **9**, 128388-16-9; BOCON, 58632-95-4; 2-bromothiophene, 1003-09-4; *N,N*-dimethylethylenediamine, 108-00-9; *N*-(2-aminoethyl)ethylenimine, 95465-58-0; *N*-(*tert*-butoxycarbonyl)ethylenediamine, 57260-73-8; 2-aminoethanol, 141-43-5; ethylenediamine, 107-15-3.