Pyrimidoquinazoline-Based Antitumor Agents. Design of Topoisomerase II to DNA Cross-linkers with Activity against Protein Kinases

Edward B. Skibo,* Xiaofen Huang, Rogelio Martinez, Robert H. Lemus, and William A. Craigo

Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287-1604

Robert T. Dorr

Arizona Cancer Center, School of Medicine, University of Arizona, Tucson, Arizona 85724

Received July 3, 2002

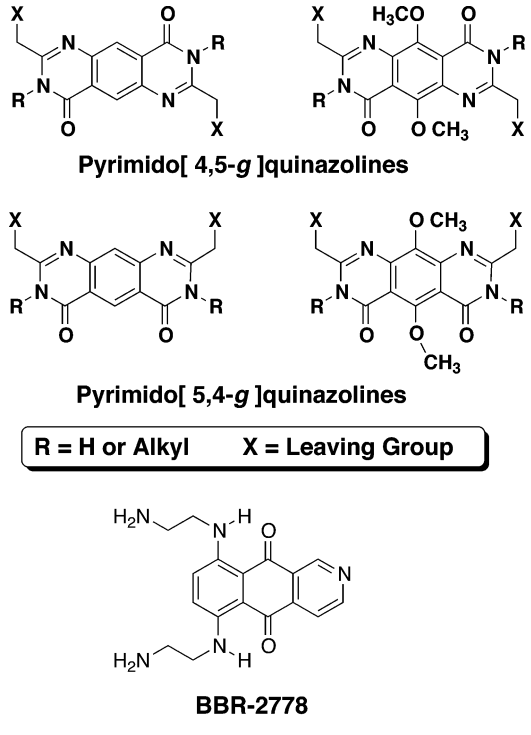
A series of pyrimidoquinazoline analogues, possessing either 4,5-*g* or 5,4-*g* fusion, were studied with respect to cytotoxicity, topoisomerase II inhibitory activity, in vivo activity, and DNA cleavage and DNA−protein cross-linking properties. These analogues were designed as electron-deficient anthraquinones with dual alkylating centers to cross-link DNA with topoisomerase II. Our studies verified the presence of DNA−protein cross-linking in vitro as well as topoisomerase II poisoning by pyrimidoquinazoline analogues. In addition, COMPARE analysis revealed that the pyrimidoquinazolines possess inhibitory activity against both topoisomerase II and protein kinases, such as the paullones, a dual property observed in other antineoplastic agents influencing phosphoester transfer.

Previous work in this laboratory involved the design of electron-deficient anthraquinone analogues based on the pyrimidoquinazoline ring system.[1,2] The analogues shown in Chart 1 were designed to intercalate DNA and then to cross-link DNA to topoisomerase II. Structural features of the pyrimidoquinazolines include a tricyclic structure bearing nitrogen and carbonyl substitution to afford an electron-deficient system, which is characteristic of a good intercalating agent.[3,4] Many of the known topoisomerase II inhibitors or intercalators are quinones: anthrapyrazoles[5] and nitrogen-substisuted anthracenediones.[6,7] An example of a clinically useful anthracenedione bearing nitrogen substitution is BBR-2778 (Chart 1).[8,9]

The pyrimidoquinazolines are essentially tetraazaanthracenediones, which are non-quinonoid (no quinone present). Another feature is the presence of two alkylation centers on all the pyrimidoquinazolines in Chart 1 as well as the presence of two electron-releasing methoxy substituents on some analogues. The methoxy substituents serve to promote the loss of leaving groups to afford alkylating carbocation species. Still another feature of the pyrimidoquinazolines is the change in relative nucleophile orientation with a change in ring fusion (4,5-*g* and 5,4-*g*). This feature was important in obtaining a structure−activity relationship for the cross-linking reaction.

This manuscript describes the synthesis, cytotoxicity assays, and mechanistic studies of cytotoxicity of the compounds in Chart 1. The results of these studies have provided a structure−activity relationship for pyrimidoquinazoline cytotoxicity and evidence that these agents cross-link DNA to topoisomerase II. COMPARE analysis of a pyrimidoquinazoline revealed a strong correlation with protein kinase inhibitors such as combretastatin[10] and a paullone analogue.[11,12] Both topoisomerase II and protein kinases are involved in phosphoester transfer to proteins, and single inhibitors could target both classes of enzymes.[13] Dual protein kinase and topoisomerase II inhibitions by the pyrimidoquinazolines could be responsible for their antitumor activity.

Chart 1



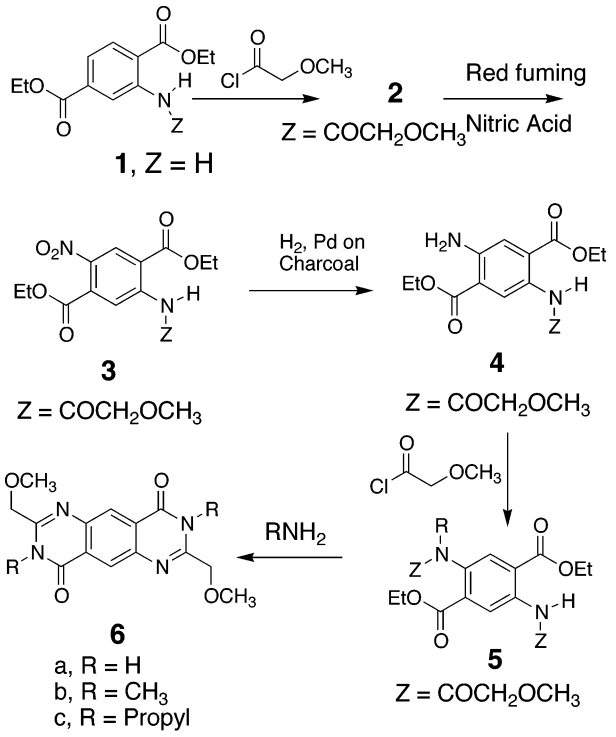
Results and Discussion

Synthesis. Shown in Schemes 1 and 2 are the synthetic steps leading to the pyrimido[4,5-*g*]quinazolines without methoxy groups on the central benzene

* To whom correspondence should be addressed. Telephone: 480-965-3581. Fax: 480-965-2747. E-mail: ESkibo@ASU.edu.

10.1021/jm020285l CCC: $22.00
Published on Web 11/05/2002

Scheme 1



ring: **8a**−**c**, **9a**,**b**, and **10**. Commercially available nitroterephthalic acid was reduced to the amine **1** and then acetylated with methoxyacetyl chloride to afford **2**. Nitration of **2** followed by reduction and acetylation steps afforded **5**, which was ring-closed to the pyrimido-[4,5-g] quinazolines **6** by treatment with primary amines. Scheme 2 shows the elaboration of the bearing groups starting with **6** (chloride, bromide, and acetate). The pyrimido[4,5-*g*]quinazolines with methoxy groups were prepared as previously described.[1]

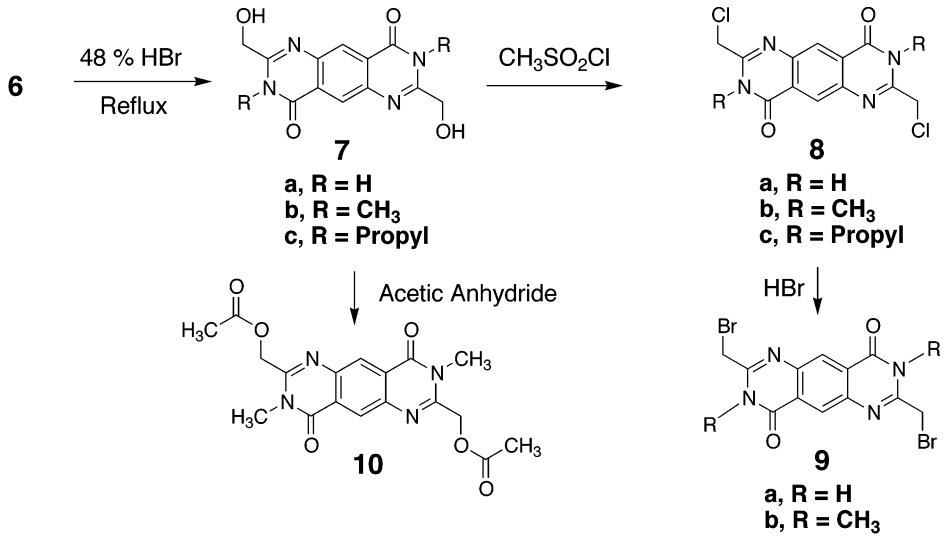
The pyrimido[5,4-*g*]quinazoline analogues possess leaving group orientations different from those of the 4,5-*g* analogues. The orientation of the alkylating centers could influence cytotoxicity, and the 5,4-*g* analogues were prepared with and without methoxy groups on the central benzene ring. Shown in Scheme 3 are the synthetic steps leading to a pyrimido[5,4-*g*]quinazoline analogue, **16**. This analogue possesses features essential for pyrimidoquinazoline cytotoxicity (R = methyl with a chloride bearing group) and was employed in comparative topoisomerase II inhibition assays with the 4,5-*g* analogue **8b.**

The starting material **11** was previously employed in the preparation of simple pyrimido[5,4-*g*]quinazolines early in the past century.[14] The conversion of **11** to **15** could not be carried out by direct coupling, and the procedure outlined in Scheme 3 had to be employed. Acetylation of **11** with methoxyacetyl chloride afforded **12**, which was ring-closed to benzoxazepine **13**. The strategy behind preparing a benzoxazepine was to block the amino group as well as to provide an ester derivative for the aminolysis step. The aminolysis reaction required the presence of NaCN,[15] which afforded the product **14** in the presence of methylamine. Deblocking of the cyanoacetamido group of **14** was readily carried out by treatment with dilute base. Finally, acetylation of **15** with chloroacetyl chloride followed by ring closure afforded the target **16.**

The synthesis of the pyrimido[4,5-*g*]quinazoline bearing methoxy groups on the central benzene ring (**24**) was carried out as outlined in Scheme 4. The 5,4-*g* analogue (**25**) shown in the inset of Scheme 4 was prepared as previously reported.[1] The annulation process started with the nitration of **19**, which was prepared from the reported compound **17**.[16] Double nitration to afford **20** resulted from the treatment with nitronium acetate (nitric acid and acetic anhydride). It was proposed that the ester groups of **20** could be converted to amides by treatment with amines. The methoxy group at the 5-position was displaced by the amine instead because of activation by the adjacent nitro groups. An alternative process involved reduction of the nitro groups to amines to afford **21** followed by aminolysis of the esters, utilizing cyanide as a catalyst. The resulting amine **22** was treated with chloroacetyl chloride to afford **23** and finally ring-closed to afford the target **24**.

To obtain water-soluble analogues of **25**, N-substituted methoxyethyl and methoxypropyl analogues (**28a**,**b**) were prepared as outlined in Scheme 5. Previously, the methyl ester of **26** could be converted to the amides by treatment with ammonia or methylamine.[1] In contrast,

Scheme 2



Scheme 3

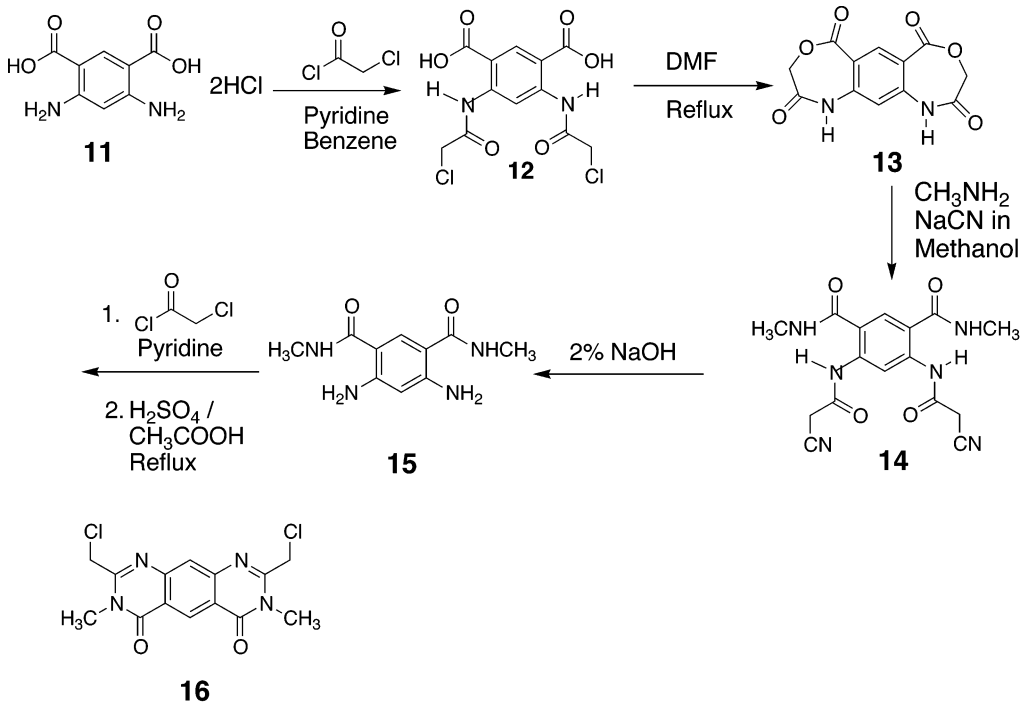


Chart 2

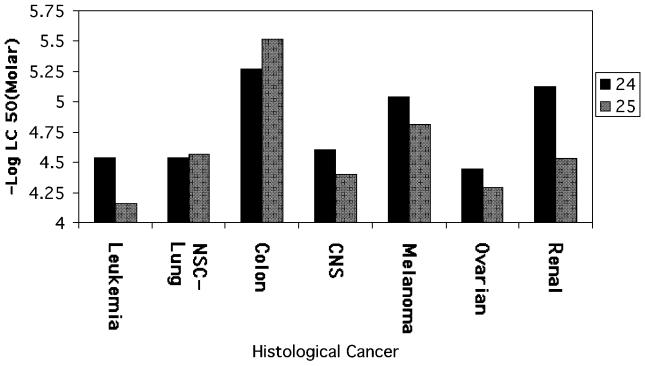


Table 1. Cytotoxicity Parameters (LC_{50}) for Pyrimidoquinazolines against Human Cancer Cell Lines

compound	cell line	LC_{50} (nM)
8b	WiDr colon	350
8b	Ovcar 3 ovarian	2000
9b	HT-29 colon	280
8a	WiDr colon	5000
9a	WiDr colon	900
9a	Ovcar 3 ovarian	850
8c	HT-29 colon	800
10b	HT-29 colon	19000
24	HT-29 colon	6330
25	HT-29 colon	110
28a	HT-29 colon	inactive
28b	HT-29 colon	inactive

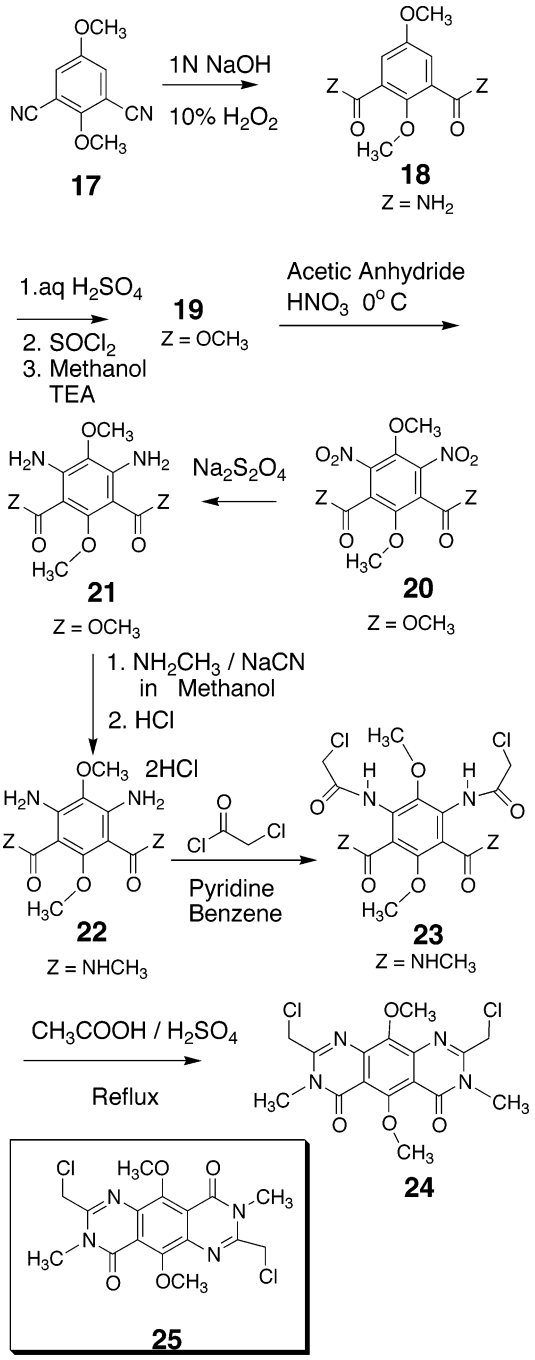
the methoxyalkylamines in Scheme 5 required the use of the acid chloride analogue to obtain the amides **27**. Preparation of the cross-linkers **28a**,**b** was carried out as previously reported.[1]

Cytotoxicity Studies. Shown in Chart 2 are the cytotoxicity data for the 4,5-*g* and 5,4-*g* fused pyrimidoquinazolines, **24** and **25**,[1] respectively, against a variety of cancer cell lines. These data were derived from the National Cancer Institute mean graph data for 60 human cancer cell lines representing major histological types (leukemia, non-small-cell lung, melanoma, etc.). For each histologic cancer type, the average −log LC_{50} value was determined from an NCI panel consisting of six to eight human cancer cell lines.[17,18] The cytotoxic parameter is the LC_{50}, which is the concentration required for 50% cell kill. The bar graph in Chart 2 reveals that ring fusion does not greatly influence the pattern and degree of cytotoxicity against major histological types of cancer. The change from 4,5-*g* to 5,4-*g* in the pyrimidoquinazoline merely changes the position of carbonyls groups while keeping the leaving groups in approximately the same position. However, removal of one of the alkylating center from the 4,5-*g* analogue (**26**)[1] resulted in a complete loss of activity in the National Cancer Institute's 60-cell line screen (data not shown).

The data in Table 1 provide insights into the structural requirements for pyrimidoquinazoline cytotoxicity. Consistent with an alkylation mechanism, excellent leaving groups such as chloride and bromide (**8b** and **9b**) are required for cytotoxicity. Accordingly, the presence of the relatively poor acetate leaving group (**10**) results in loss of cytotoxicity. The central methoxy groups of **25** increased cytotoxicity greatly, presumably because they assist in leaving group loss during the alkylation process by facilitating full or partial carbocation formation. The nitrogen substituents influence cytotoxicity with *N*-methyl analogues that are more active than N-unsubstituted analogues in terms of both cytotoxicity and solubility. The N-unsubstituted analogues may be too polar to enter cells with facility. However, increasing the N-substituent size to propyl (**8c**) resulted in loss of cytotoxicity.

Design of Water-Soluble Pyrimidoquinazolines. The *N*-methyl-substituted pyrimidinoquinazolines are not soluble in aqueous buffers, and in vivo studies could not be carried out. To increase water solubility, analogues bearing methoxyalkyl substituents (**28a**,**b**) were

Scheme 4



prepared and evaluated in vitro and in vivo. The new pyrimidoquinazolines were in fact soluble in water but displayed low activity in the NCI's in vitro screen. Only **28a** possessed moderate in vivo activity in the B16 melanoma syngraft assay[19] (Figure 1). A dosage of 1, 2, and 4 mg/kg was given on days 1, 5, and 9 after subcutaneous implantation of 10^5 cells in the front flank on day 0. The control group and the three dosage groups each consisted of four mice. The average tumor mass of the control and 1 mg/kg groups was statistically the same at the end of 28 days, while the 2 and 4 mg/kg groups were able to reduce the tumor mass by about 50%. The moderate in vivo activity of **28a** confirms that water-soluble active analogues of the pyrimidoquinazolines can be designed, although it is clear that more work needs to be done in this area.

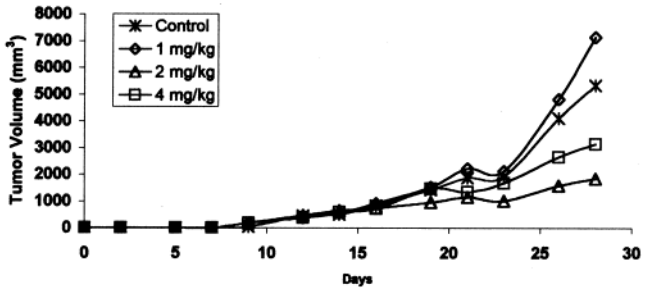


Figure 1. Results of pyrimidoquinazoline **28a** in the B-16 melanoma model in C57/bl mice (run at University of Arizona Cancer Center). This compound was studied at three dose levels: 1, 2, and 4 mg/kg ip on days 1, 5, and 9 after tumor implantation into the front flank muscle. Shown in the plot are the area-under-the curve values as a function of time for each dosage. The control was obtained with drug-free animals.

COMPARE Analysis. COMPARE was developed at the National Cancer Institute to compare the patterns of cytotoxicity in their 60-cell line cancer panel.[17] Antitumor agents with identical mechanisms of action possess identical or nearly identical cytotoxicity patterns (correlation coefficient greater than 0.8). The growth inhibition (GI_{50}) and total growth inhibition (TGI) profiles of **24** (NSC 676424) were compared with those of known antitumor agents in the NCI archives. High correlations were observed with the agents whose structures and NSC numbers are shown in Chart 3.

The GI_{50} profile of **24** correlated highly (correlation coefficients of 0.62−0.74) with a series of chalcone derivatives represented by compound 657747 in Chart 3. The combretastatin derivative 615258[10,20,21] correlates with the GI_{50} profile of **24** with a coefficient of 0.610. Both the stilbene and chalcone[22] structural elements are typical of antitubulin compounds that inhibit protein kinases.[23] These structural elements, highlighted in red, are also present in the indole derivative 676870 (correlation coefficient of 0.66) and the brominated paullone derivative 673433 (correlation coefficient of 0.6). The paullones are small-molecule inhibitors of tyrosine kinases including the CDKs.[11] The topoisomerase II inhibitor that shows the highest correlation with the GI_{50} profile of **24** is azotoxin[24] with a correlation coefficient of 0.62. It is noteworthy that azotoxin also possesses antitubulin properties along with the stilbene-like structural element shown in red. The TGI profile of **24** correlated highly with compound 667262 (correlation coefficient of 0.6), which possesses chalcone-like structural features

The COMPARE results presented above suggest that pyrimidoquinazolines possess inhibitory activity against both topoisomerase II and protein kinases, a dual property observed in other antineoplastic agents.[13] Since pyrimidoquinazoline **24** does not possess either the chalcone or stilbene structural elements of the protein kinase inhibitors shown in Chart 3, the high correlation coefficients obtained from the COMPARE analysis were surprising. However, the pyrimidoquinazoline **24** does possess fused pyrimidine rings, a structural feature found in many other classes of protein kinase inhibitors.[25−28]

Topoisomerase II Inhibition Assays. Relaxation assays were carried out with recombinant human topoisomerase II, and the completed reactions were

Scheme 5

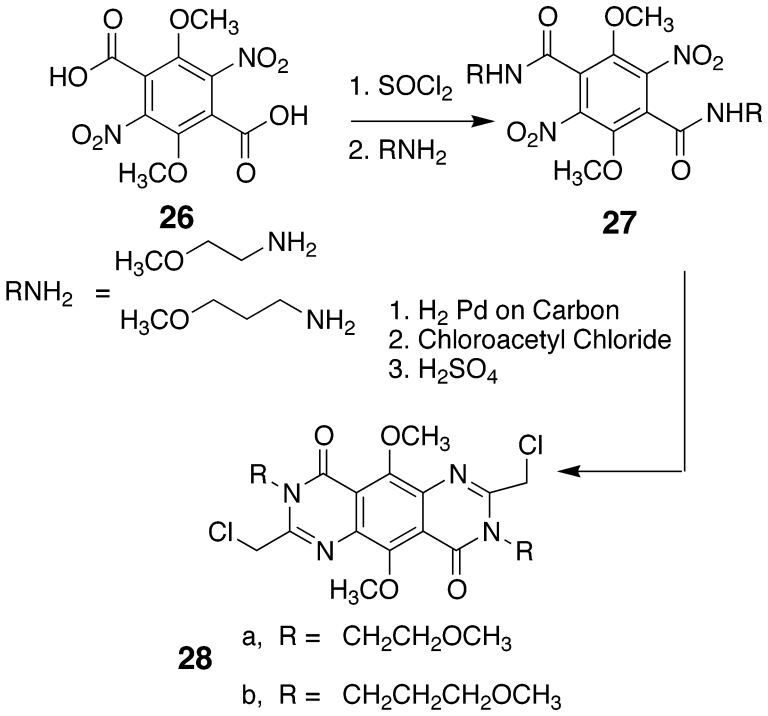
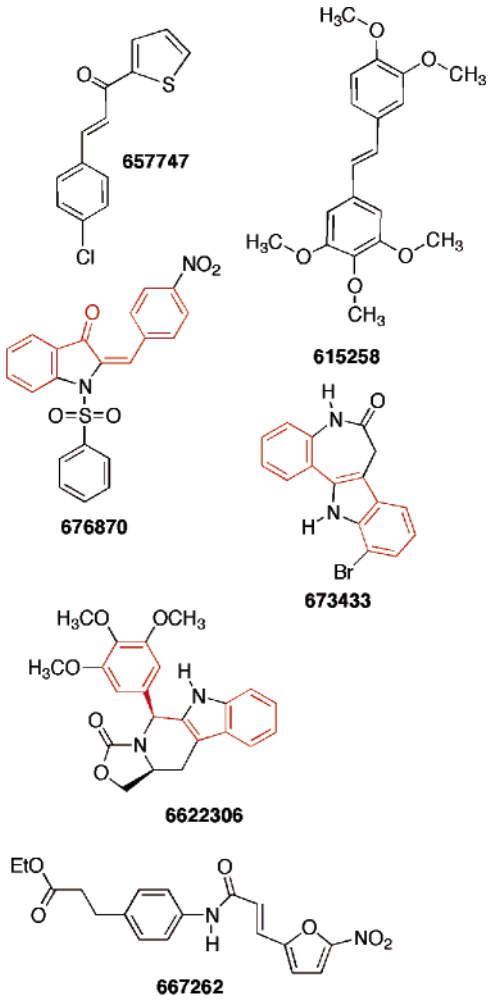


Chart 3



assayed on agarose gels run with or without ethidium bromide. The EB-containing gels will readily detect the presence of linear DNA but will hardly resolve form I and form II DNAs (form II traveled slightly more quickly than form I). If linear DNA is present, then the agent acts as a topoisomerase II poison; i.e., the agent stabilizes the cleavable complex. In contrast, non-EB-containing gels will readily resolve the form II topomers arising from the supercoiled or form I DNA and catalytic inhibition could then be documented by noting the decrease in relaxation with increasing inhibitor concentration.

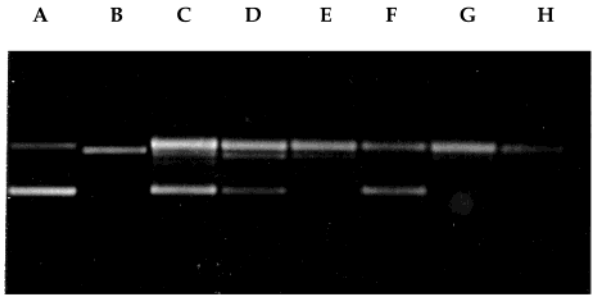


Figure 2. Agarose gels of topoisomerase II catalyzed relaxations of of pRYG supercoiled DNA (form I along with supercoiled dimer and trimer) run in the absence of ethidium bromide. The control lanes include unrelaxed pRYG supercoiled DNA in lane A, the linear form of pRYG supercoiled DNA in lane B, and lanes C and D are relaxation reactions carried out with and without DMSO. Lanes E and F are relaxation reactions carried out in the presence of 0.25 and 0.5 mM of **9b**, and lanes G and H likewise are carried out in the presence of 0.25 and 0.5 mM **9a**. Compound **9b** shows significant inhibition compared to **9a**.

Shown in Figures 2 and 3 are the assays for the relaxation of pRYG supercoiled DNA by p170 human topoisomerase II in the presence of pyrimidoquinazoline derivatives. In Figure 2, a comparison is made between pyrimidoquinazolines **9a** and **9b** in order to assess the importance of the *N*-methyl group. The first four lanes are controls showing supercoiled DNA (lane A), linear

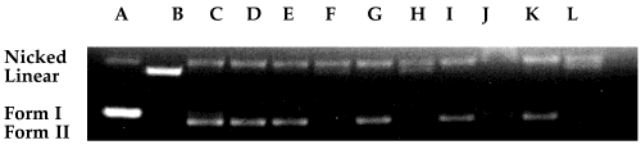


Figure 3. Agarose gels of topoisomerase II catalyzed relaxations of of pRYG supercoiled DNA (form I along with supercoiled dimer and trimer) run in the presence of ethidium bromide. The control lanes (A−D) are the same as described in the Figure 2 caption. Lanes E and F are relaxation reactions carried out in the presence of 0.25 and 0.75 mM of **16**, and lanes G and H likewise are carried out in the presence of 0.25 and 0.75 mM **8b**. Lanes I and J are relaxation reactions carried out in the presence of 0.25 and 0.75 mM of **24**, and lanes K and L likewise are carried out in the presence of 0.25 and 0.75 mM **25**.

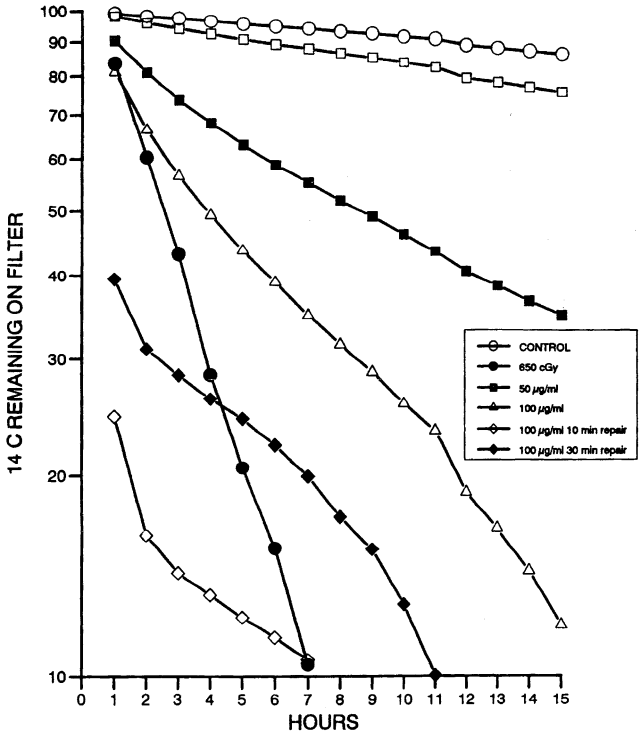


Figure 4. Single-strand-break alkaline elution assay for **25** in L 1210 leukemia.

DNA (lane B), and relaxation with and without DMSO (lanes C and D, respectively). Lanes E and F show relaxation reactions in the presence **9b**, and lanes G and H show relaxation reactions in the presence **9a**. The N-methylated analogue **9b** inhibits topoisomerase II mediated relaxation, while **9a** does not inhibit relaxation at the concentrations tested. This result parallels the cytotoxicity data enumerated in Table 1 wherein N-methylated analogues are much more active than N-unsubstituted analogues. The EB-containing gel in Figure 3 shows linear DNA formation during relaxation reactions in the presence cross-linkers **16** (lanes E and F), **8b** (lanes E and F), **24** (lanes E and F), and **25** (lanes E and F). In addition, none of the forms I and II DNA is present in reactions with the higher concentration (0.75 mM) of cross-linker. These results are consistent with a mechanism involving stabilization of the topoisomerase II−DNA cleavable complex by the cross-linker followed by linearization of the supercoiled DNA upon proteinase treatment. The presence of the processes of protein (topoisomerase II)−DNA cross-linking and single

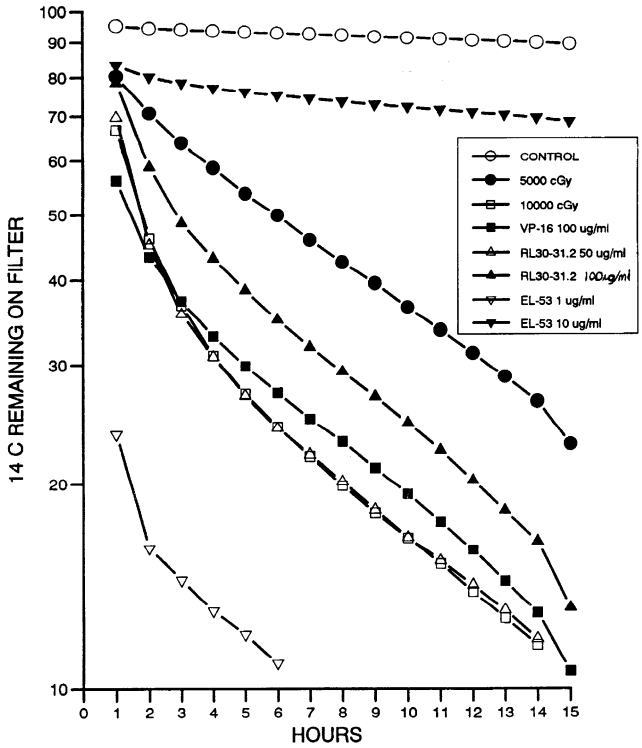


Figure 5. Double-strand-break alkaline elution assay for **25** in L 1210 leukemia.

or double strand DNA cleavage were verified in vitro by the alkaline elution assays discussed below.

Alkaline Elution Assays. These assays were carried out in L 1210 leukemia cells with pyrimidoquinazoline **25** in order to verify the presence of DNA cleavage and cross-linking processes in vitro.

The single strand break assay results are shown in Figure 4. The control curve shows the slow elution of unbroken DNA, while treatment with 650 cGy of radiation shows the rapid elution of single strand cleaved DNA. Addition of 50 and 100 μg/mL of **25** (RL-30-31.2) resulted in increased single strand cleavage (more rapid elution of DNA with increasing drug concentration). Removal of the drug from the cell culture by washing did not result in repair, which is seen in many topoisomerase poisons. DNA repair is an important (but not the only)[29,30] resistance mechanism for topoisomerase II poisons such as the anthracyclines.[31]

The double strand break alkaline elution assay results are shown in Figure 5. Positive controls, which cause double strand cleavage, are treated with 5000 and 10 000 cGy of radiation and VP-16 (etopside). The rapid elution of DNA in cells treated with **25** (RL-30-31.2) indicates that double strand cleavage of DNA (equivalent to that seen with etopside) has taken place. Also shown is an anthracenedione topoisomerase II inhibitor, EL-53, that also causes double strand breaks.

The protein−DNA cross-linking alkaline elution assay results are shown in Figure 6. The control and 30 cGy treated cells show slow and rapid elution of DNA, respectively. Treatment of cells with **25** (RL-30-31.2) followed by 3000 cGy of radiation to induce strand breaks resulted in a slow elution phase after 5−6 h due to protein-DNA cross-links. The anthracenedione EL-53 had a much lower retention of DNA than the cross-linker **25**.

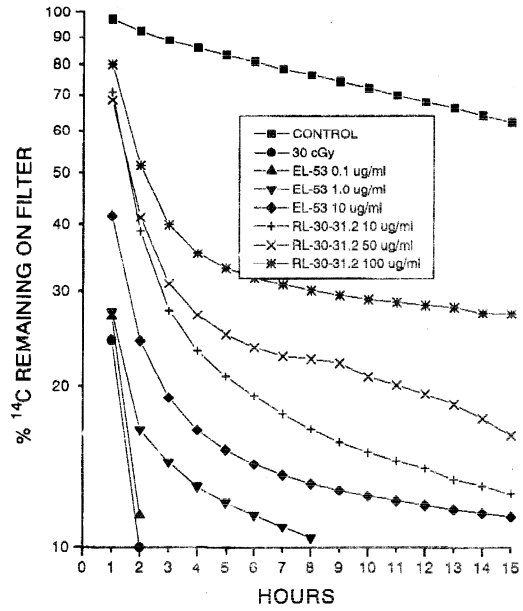


Figure 6. DNA−protein cross-linking alkaline elution assay for **25** in L 1210 leukemia.

The results described above indicate that **25** causes single and double strand DNA breaks as well as DNA−protein cross-linking.

Conclusions

The design of electron-deficient anthraquinone analogues based on the pyrimidoquinazoline ring system has been carried out, and a structure−activity relationship and mechanism of cytotoxicity have been elucidated. From these studies, the following conclusions were made.

The ring fusion of the pyrimidoquinazoline (4,5-*g* and 5,4-*g*) does not greatly influence cytotoxicity. Our explanation for this observation is that changing of the ring fusion does not greatly change the position of the alkylating centers. The 4,5-*g* fused analogues are easier to prepare and will be the subject of further development.

The presence of two alkylating centers is vital for activity because cytotoxicity relies on a cross-linking process. Alkaline elution assays indicate that DNA is linked to a protein, perhaps topoisomerase II. The single strand break alkaline elution assay indicated the absence of DNA repair typically seen when the topoisomerase inhibitor is removed from the cell culture. The linking of DNA to a large structure would in fact preclude any repair of a strand break.

The presence of methoxy groups on the central benzene ring of the pyrimidoquinazoline ring confers only a slight increase in cytotoxicity (3-fold) over unsubstituted analogues (compare **8b** with **25** in Table 1). Development of pyrimidoquinazoline analogues with an unsubstituted central benzene ring is preferred over the methoxy derivatives because of their relative ease of synthesis.

The N substituent greatly influences cytotoxicity, which varies from the inactive N-unsubstituted analogues to the highly active *N*-methyl analogues. The solubility of these analogues no doubt plays a role in cytotoxicity. While the N-unsubstituted analogues are completely insoluble because of symmetry and hydrogen-bonding interactions, the *N*-methyl analogues are slightly more soluble because of the elimination of the hydrogen-bonding interactions. In vivo studies were not possible with either the N-unsubstituted or *N*-methyl analogues. However, we prepared an *N*-(2-methoxyethyl) analogue that was soluble enough in aqueous media to exhibit good in vivo activity in the B16 melanoma model.

COMPARE studies on the pyrimidoquinazolines revealed high correlations with both topoisomerase II and protein kinase inhibitors. This dual property, observed in other antineoplastic agents, suggests that pyrimidoquinazolines should be the subject of further study.

Experimental Section

All analytically pure compounds were dried under high vacuum in a drying pistol over refluxing toluene. Elemental analyses were run at Atlantic Microlab, Inc., Norcross, GA. All TLC measurements were performed on silica gel plates using a variety of solvents and a fluorescent indicator for visualization. IR spectra were taken as thin films, and the strongest absorbances were reported. ^{1}H NMR spectra were obtained from a 300 MHz spectrometer. All chemical shifts are reported relative to TMS.

2-Aminoterephthalic Acid Diethyl Ester (1). To a solution of 3.0 g of 2-nitroterephthalic acid in 60 mL of absolute ethanol was added 5 mL of concentrated sulfuric acid with cooling. The reaction mixture was then refluxed for 24 h, after which time the excess ethanol was removed in vacuo. The residue was combined with 100 mL of water, and the resulting mixture was extracted with 2 × 50 mL portions of chloroform. The chloroform extracts were washed with 4 × 50 mL portions of saturated aqueous sodium bicarbonate and then with 2 × 50 mL of water. Drying the washed extracts (Na_2SO_4) was followed by evaporation in vacuo to an oil, which crystallized upon addition of hexane. Recrystallization of the solids from ethyl acetate/hexane afforded pure 2-nitroterephthalic acid diethyl ester: 2.25 g (59%) yield; mp 52−53 °C (lit. mp 57 °C).

A mixture of 2.0 g (7.48 mmol) of the above product, 150 mg of 5% Pd on carbon, and 35 mL of ethanol was shaken under 50 psi of H_2 for 4 h. The reaction mixture was filtered through Celite, and the filtrate was then concentrated to an oil, which crystallized on standing. Recrystallization of the solids from ethyl acetate/hexane afforded pure **1**: 1.5 g (85%) yield; mp 56−57 °C; TLC (ethyl acetate/benzene [2:3]), R_f = 0.58; IR (KBr pellet) 3449, 2891, 1722, 1282, 1112 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.05 and 7.71 (2H, ABX, J_{ortho} = 8.3 Hz, J_{meta} = 1.6 Hz, $J_{para} \approx$ 0 Hz, C(6) and C(5) aromatic protons, respectively), 7.99 (1H, brs, C(3) aromatic proton), 4.38 (4H, 2q, ester methylenes), 1.38 (6H, 2t, ester methyls); mass spectrum (EI mode), *m*/*z* 237 (M^+), 191 ($M^+ - C_2H_5OH$).

2-(Methoxyacetamido)terephthalic Acid Diethyl Ester (2). To a solution of 5.0 g (21 mmol) of **1** in 100 mL of benzene were added 1.9 mL (23 mmol) of pyridine and 2.1 mL (23 mmol) of methoxyacetyl chloride. The reaction mixture was stirred at room temperature for 2 h and then extracted with 2 × 50 mL of water. Drying the extracts (Na_2SO_4) was followed by concentration in vacuo to an oil, which crystallized during trituration with hexane: 5.25 g (80%) yield; mp 74−75 °C; TLC (ethyl acetate/benzene [1:4]), R_f = 0.39; IR (KBr pellet) 1719, 1697, 1576, 1525, 1420, 1287, 1265, 1229 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.36 (1H, ABX, d, J_{meta} = 1.7 Hz, C(3) aromatic proton), 8.09 and 7.74 (2H, ABX, J_{ortho} = 8.3 Hz, J_{meta} = 1.7 Hz, $J_{para} \approx$ 0 Hz, C(6) and C(5) aromatic protons, respectively), 4.39 (4H, 2q, ester methylenes), 4.06 (2H, s, acetamido methylene), 3.54 (3H, s, acetamido methyl), 1.39 (6H, 2t, ester methyls); mass spectrum (EI mode), *m*/*z* 308 (M^+), 279 ($M^+ -$ HCHO), 264 ($M^+ - OC_2H_5$). Anal. ($C_{15}H_{19}NO_6$) C, H, N.

2-(Methoxyacetamido)-5-nitroterephthalic Acid Diethyl Ester (3). To 25 mL of red fuming nitric acid, chilled to 0−5 °C with an ice bath, was added 3.8 g (12 mmol) of **2** portionwise. The reaction mixture was maintained below 10

°C during the addition, after which the reaction mixture was stirred without ice bath cooling for 30 min. After the reaction was completed, the mixture was poured over 130 g of crushed ice and then extracted with 3 × 35 mL portions of chloroform. Drying the chloroform extracts (Na_2SO_4) was followed by concentration in vacuo to an oil, which crystallized upon addition of ethanol. Recrystallization from aqueous ethanol afforded pure **3**: 3.14 g (72%) yield; mp 86−88 °C; TLC (ethyl acetate/benzene [1:4]), $R_f = 0.46$; IR (KBr pellet) 1735, 1706, 1573, 1535 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.10 and 8.76 (2H, 2s, C(3) and C(6) aromatic protons), 4.43 (4H, 2q, ester methylenes), 4.07 (2H, s, acetamido methylene), 1.43 and 1.35 (6H, 2t, ester methyls); mass spectrum (EI mode), *m*/*z* 354 (M^+), 324 ($M^+ - NO$), 309 ($M^+ - CH_2OCH_5$). Anal. ($C_{15}H_{18}N_2O_8$) C, H, N.

2-(Methyoxyacetamido)-5-aminoterephthalic Acid Diethyl Ester (4). A mixture consisting of 1.0 g (2.82 mmol) of **3**, 100 mg of 5% Pd on carbon, and 100 mL of absolute ethanol was shaken under 50 psi of H_2 for 1.5 h. The reaction mixture was then filtered through Celite, and the filtrate was concentrated in vacuo. The resulting yellow solid was recrystallized from ethyl acetate/hexane: 750 mg (82%) yield; mp 158−160 °C; TLC (ethyl acetate/benzene [1:1]), $R_f = 0.51$; IR (KBr pellet) 3460, 3337, 1685, 1666, 1576, 1536, 1425, 1232, 1205, 1107 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.18 and 7.35 (2H, 2s, C(3) and C(6) aromatic protons), 5.60 (2H, brs, amino protons), 4.35 (4H, 2q, ester methylenes), 4.02 (2H, s, acetamido methylene), 3.51 (3H, s, acetamido methyl), 1.38 (6H, 2t, ester methyls); mass spectrum (EI mode), *m*/*z* 324 (M^+), 279 ($M^+ - OC_2H_5$). Anal. ($C_{15}H_{20}N_2O_6$) C, H, N.

2,5-Bis(methoxyacetamido)terephthalic Acid Diethyl Ester (5). To a solution of 1.5 g (4.64 mmol) of **4** in 100 mL of dry benzene were added 410 μL (5.07 mmol) of dry pyridine and then 470 μL (5.14 mmol) of methoxyacetyl chloride. The mixture was stirred for 2 h and then diluted with 200 mL of chloroform. Extraction of the diluted reaction mixture with 2 × 100 mL portions of water was followed by drying of the organic layer (Na_2SO_4) and concentration of this layer in vacuo. The resulting oily residue was crystallized by dissolution in a small volume of chloroform followed by addition of hexane: 1.7 g (91%) yield; mp 191−192 °C; TLC (ethyl acetate/benzene [1:1]), $R_f = 0.43$; IR (KBr pellet) 1701, 1675, 1549, 1404, 1316, 1272, 1252 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.44 (2H, s, C(3) and C(6) aromatic protons), 4.41 (4H, q, $J = 7.2$ Hz ester methylenes), 4.04 (4H, s, acetamido methylenes), 3.53 (6H, s, acetamido methyls), 1.40 (6H, t, $J = 7.2$ Hz, ester methyls); mass spectrum (EI mode), *m*/*z* 396 (M^+), 351 ($M^+ - OC_2H_5$). Anal. ($C_{18}H_{24}N_2O_8$) C, H, N.

2,7-Bis(methoxymethyl)pyrimido[4,5-*g*]quinazoline-4,9-(3*H*,8*H*)-dione (6a). 6a was prepared in two steps from **5** as described below.

To a suspension of 500 mg (1.26 mmol) of **5** in 20 mL of methanol was added 9.8 mL of hydrazine hydrate. The reaction mixture was stirred for 18 h, and the crystallized product was then removed by filtration and washed with ethanol: 385 mg (92%) yield. Pure 2,7-bis(methoxymethyl-3,8-diaminopryimido-[4,5-*g*quinazoline-4,9-(3*H*,8*H*)-dione was obtained by recrystallization from dimethyl sulfoxide: mp 258−259 °C (dec); TLC (1-butanol/acetic acid/water [5:2:3]), $R_f = 0.66$; IR (IBr pellet) 3186, 1666, 1630, 1612, 1455, 1126, 1083, 980, 842, 786 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 8.32 (2H, s, C(5) and C(10) aromatic protons), 5.65 (4H, s, N(3) and N(8) amino protons), 4.66 (4H, s, methoxymethylmethylenes), 3.44 (6H, s, methoxys); mass spectrum (EI mode), *m*/*z* 332 (M^+), 302 ($M^+ -$ HCHO), 272 ($M^+ -$ 2HCHO).

To a suspension consisting of 500 mg (1.50 mmol) of the product obtained above in 15 mL of glacial acetic acid was added 2.0 g of KNO_3. After the reaction mixture was heated at reflux for 2 min, the reaction mixture was cooled and then the KNO_3 addition−heating−cooling sequence was repeated four more times. Addition of 75 mL of water to the completed reaction precipitated the product, which was filtered off and washed with water: 397 mg (87%) yield. Recrystallization from dimethyl sulfoxide afforded pure **6a**: mp >250 °C; TLC (1-butanol/acetic acid/water [5:2:3]), $R_f = 0.61$; IR (KBr pellet) 3180, 3070, 2943, 1673, 1624, 1464, 1127, 1118 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 8.29 (2H, s, C(5) and C(10) aromatic protons), 4.37 (4H, s, methoxymethylmethylenes), 3.39 (6H, s, methoxys); mass spectrum (EI mode), *m*/*z* 302 (M^+) 272 ($M^+ -$ HCHO), 242 ($M^+ -$ 2HCHO).

2,7-Bis(methoxymethyl)-3,8-dimethylpyrimido[4,5-*g*]-quinazoline-4,9-(3*H*,8*H*)-dione (6b). A solution of 500 mg (1.26 mmol) of **5** in 50 mL of 20% (w/w) methylamine in dimethylformamide was heated at 120 °C in a sealed volume in vacuo and resulted in crystallization of the product, which was filtered off and washed with acetone. Recrystallization from dimethylformamide afforded **6b** as off-white needles: 351 mg (84%) yield; mp 270−271 °C; TLC (chloroform/methanol [9:1]), $R_f = 0.63$; IR (KBr pellet) 1688, 1606, 1452, 1421, 1380, 1340, 1091, 957, 815, 798 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 8.30 (2H, s, C(5) and C(10) aromatic protons), 4.58 (4H, s, C(2) and C(7) methoxymethylmethylenes), 3.55 (6H, s, N(3) and N(8) methyls), 3.37 (6H, s, methoxys); mass spectrum (EI mode), *m*/*z* 330 (M^+), 300 ($M^+ -$ HCHO). Anal. ($C_{16}H_{18}N_4O_4$) C, H, N.

2,7-Bis(methoxymethyl)-3,8-bis(*n*-propyl)pyrimido[4,5-*g*]quinazoline-4,9-(3*H*,8*H*)-dione (6c). 6c was prepared in two steps from **5** as described below.

A mixture consisting of 344 mg (0.94 mmol) of **5**, 100 mg of powdered NaCN, and 2.5 mL of *n*-propylamine was stirred for 8 h. After this time, water was added to the reaction mixture, resulting in crystallization of pure 2,5-bis(methoxyacetamido)-1,4-bis(*n*-propylcarbamyl)benzene: 265 mg (66%) yield; mp 254−255 °C; TLC (chloroform/methanol [9:1]), $R_f = 0.72$; ^1H NMR (dimethyl sulfoxide-d_6) δ 11.76 (2H, s, methoxyacetamido N−H proton), 8.83 (2H, t, $J = 54.4$ Hz, carbamyl N−H proton split by propylmethylene), 8.68 (2H, s, aromatic), 4.00 (4H, s, methoxymethylmethylenes), 3.41 (6H, s, methoxys), 3.22 (4H, q, $J = 6.1$ Hz, propylmethylenes adjacent to N), 1.54 (4H, sextet, $J = 7$ Hz, other propylmethylenes), 0.91 (6H, t, $J = 7.3$ Hz, propylmethyls); mass spectrum (EI mode) *m*/*z* 422 (M^+).

A mixture of 56 mg of the product obtained above, 200 μL of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and 3 mL of dry dimethylformamide was heated at 140 °C for 3 h. The product began to crystallize from solution during heating; crystallization was completed by chilling the reaction mixture in a refrigerator for several hours. The crystalline product was filtered, washed with methanol, and dried: 37 mg (72%) yield; mp 180−181 °C; TLC (chloroform/methanol [9:1]), $R_f = 0.74$; mass spectrum (EI mode) *m*/*z* 386 (M^+). Anal. ($C_{20}H_{26}N_4O_4$) C, H, N.

2,7-Bis(hydroxymethyl)pyrimido[4,5-*g*]quinazoline-4,9-(3*H*,8*H*)-dione (7a). A suspension of 304 mg (1.01 mmol) of **6a** in 55 mL of 48% aqueous hydrobromic acid was refluxed for 50 h. The reaction mixture was then cooled to room temperature, and the crystallized product was filtered off. The product was washed with ethyl acetate and then with ethanol: 247 mg (89%) yield. Purification was carried out by dissolution of the product in dimethyl sulfoxide and then precipitation by addition of methanol: mp >250 °C (dec); TLC (2-propanol/water/ammonium hydroxide [7:2:1]), $R_f = 0.69$; IR (KBr pellet) 3292, 2796, 1728, 1718, 1638, 1118, 1572, 1484, 1345, 1277 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 8.40 (2H, s, C(5) and C(10) aromatic protons), 4.51 (4H, s, C(2) and C(7) hydroxymethyls); mass spectrum (EI mode), *m*/*z* 272 (M^+).

2,7-Bis(hydroxymethyl)-3,8-dimethylpyrimido[4,5-*g*]-quinazoline-4,9-(3*H*,8*H*)-dione (7b). A suspension of 500 mg (1.51 mmol) of **6b** in 50 mL of 48% hydrobromic acid was refluxed for 3.5 h. The reaction mixture was cooled, and the precipitated product was filtered off. The product was washed with ethyl acetate and then with ethanol: 385 mg (84%) yield; mp >230 °C (dec); TLC (1-butanol/acetic acid/water [5:2:3]), $R_f = 0.55$; IR (KBr pellet) 3203, 3045, 3013, 3002, 1722, 1627, 1559, 1308, 1096, 774 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 8.61 (2H, s, C(5) and C(10) aromatic protons), 4.79 (4H, s, C(2) and C(7) hydroxymethylmethylenes), 3.59 (6H, s, N(3) and N(4) methyls); mass spectrum (EI mode), *m*/*z* 302 (M^+).

2,7-Bis(hydroxymethyl)-3,8-bis(*n*-propyl)pyrimido[4,5-*g*]quinazoline-4,9-(3*H*,8*H*)-dione (7c). A suspension of 218 mg (0.56 mmol) of **6c** in 10 mL of 48% aqueous hydrobromic acid was refluxed for 12 h. The reaction mixture was then chilled, and the pH was adjusted to 7 with saturated sodium bicarbonate. The crystallized product was filtered off, washed with water, and dried: 151 mg (75%) yield; mp 205−215 °C (dec); TLC (ethyl acetate), R_f = 0.36; IR (KBr pellet) 3387, 2964, 2877,1680, 1604, 1467, 1379, 1356, 1261, 1099, 1068, 796 cm^{-1}; ^{1} NMR (dimethyl sulfoxide-d_6) δ 8.35 (2H, s, C(5) and C(10) aromatic protons), 5.80 (2H, brs, hydroxyl protons), 4.65 (4H, s, hydroxymethylmethylenes), 4.075 (4H, t, J = 5.7 Hz, propylmethylenes adjacent to N0, 1.76 (4H, sextet, $J \approx 6$ Hz, other propylmethylenes), 0.96 (6H, t, J = 7.4 Hz, propylmethyls); mass spectrum (EI mode), m/z 358 (M^+), 328 ($M^+ - CH_2O$), 298 ($M^+ - 2CH_2O$), 285 ($M^+ - CH_2O$-propyl).

2,7-Bis(chloromethyl)pyrimido[4,5-*g*]quinazoline-4,9-(3*H*,8*H*)-dione (8a). To an ice bath chilled mixture consisting of 104 mg (0.379 mmol) of **7a**, 960 μL (12 mmol) of pyridine, and 35 mL of dimethylformamide was added 960 μL (12 mmol) of methanesulfonyl chloride. The reaction mixture was maintained at 5 °C during the addition and for 20 min thereafter. The reaction mixture was then allowed to warm to room temperature and stirred for 2.5 h. Water (150 mL) was added to the reaction mixture after this period of time, and the resulting mixture was stirred for 2 h. The precipitated product was filtered off and dried: 61 mg (52%) yield. Purification was carried out by dissolution of the product in a small volume of dimethyl sulfoxide followed by precipitation with methanol: mp >200 °C (dec); TLC (ethyl acetate/methanol [9:1]), R_f = 0.48; IR (KBr pellet) 3112, 3061, 3010, 2947, 1672, 1616, 1493, 1461, 1420, 1278 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 8.32 (2H, s, C(5) and C(10) aromatic protons), 4.59 (4H, s, C(2) and C(7) chloromethyls); mass spectrum (EI mode), m/z 310 (M^+, $^{35}Cl^{35}Cl$), 312 (M^+, $^{35}Cl^{37}Cl$), 314 (M^+, $^{37}Cl^{37}Cl$). Anal. ($C_{12}H_8Cl_2N_4O_2$) C, H, N.

2,7-Bis(chloromethyl)-3,8-dimethylpyrimido[4,5-*g*]quinazoline-4,9-(3*H*,8*H*)-dione (8b). 8b was prepared and isolated in crude form (28% yield) by the procedure described for the preparation of **8a**.

The pale-yellow product was flash-chromatographed on a column of silica gel using chloroform/methanol (99:1) as eluant. The purified product was recrystallized from chloroform/hexane: mp >210 °C (dec); TLC (chloroform/methanol [9:1]), R_f = 0.66; IR (KBr pellet) 1688, 1669, 1604, 1470, 1454, 1421, 1342, 816, 798 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 8.34 (2H, s, C(5) and C(10) aromatic protons), 4.95 (4H, s, C(2) and C(7) chloromethyls), 3.66 (6H, s, N(3) and N(8) methyls); mass spectrum (EI mode) m/z 338 (M^+, $^{35}Cl^{35}Cl$), 340 (M^+, $^{37}Cl^{35}Cl$), 342 (M^+, $^{37}Cl^{37}Cl$). Anal. ($C_{14}H_{12}Cl_2N_4O_2 \cdot 0.2H_2O$) C, H, N.

2,7-Bis(chloromethyl)-3,8-bis(*n*-propyl)pyrimido[4,5-*g*]quinazoline-4,9-(3*H*,8*H*)-dione (8c). A mixture consisting of 56 mg (0.156 mmol) of **7c**, 200 μL of methanesulfonyl chloride, 200 μL of pyridine, and 1 mL of dry dimethyl formamide was heated at 80 °C until complete dissolution occurred (about 2 min). The reaction mixture was then cooled to room temperature, resulting in crystallization of **8c**. The pale product was flash-chromatographed on a silica gel column employing chloroform/methanol (98:2) as eluent. The purified product was recrystallized from chloroform/hexane: mp 219 °C (dec); TLC (chloroform/methanol [9:1]), R_f =0.71; IR (KBr pellet) 1686, 1591, 1469, 1377 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.60 (2H, s, C(5) and C(10) aromatic protons), 4.64 (4H, s, C(2) and C(7) chloromethyls), 4.16 (4H, q, J = 5.7 Hz, propylmethylenes adjacent to N), 1.81 (4H, sextet, J = 7.7 Hz, other propylmethylenes), 1.04 (6H, t, J = 7.5 Hz, propylmethyls); mass spectrum (EI mode), m/z 394(M^+, $^{35}Cl^{35}Cl$), 396 (M^+, $^{37}Cl^{35}Cl$), 398 (M^+, $^{37}Cl^{37}Cl$). Anal. ($C_{18}H_{20}Cl_2N_4O_2$) C, H, N.

2,7-Bis(bromomethyl)pyrimido[4,5-*g*]quinazoline-4,9-(3*H*,8*H*)-dione (9a). To a stirred suspension of 10 mg (0.032 mmol) of **8a** in 2.0 mL of dimethyl sulfoxide was added 270 μL of 48% aqueous hydrobromic acid. After the reaction mixture was stirred for 40 min, the product was precipitated as a pale-yellow solid, 4.9 mg (38%) yield, by adding 40 mL of water. Purification was carried out by precipitating the product from a concentrated dimethyl solution with water: mp >250 °C (dec); TLC (ethyl acetate/methanol [9:1]), R_f = 0.50; IR (KBr pellet) 3035, 2947, 1681, 1616, 1461, 1108, 1093, 798 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 8.29 (2H, s, C(5) and C(10) aromatic protons), 4.44 (4H, s, C(2) and C(7) bromomethyls); mass spectrum (EI mode), m/z 398 (M^+, $^{79}Br^{79}Br$), 400 (M^+, $^{79}Br^{81}Br$), 402 (M^+, $^{81}Br^{81}Br$). Anal. ($C_{12}H_6Br_2N_4O_4 \cdot 0.75H_2O$) C, H, N.

2,7-Bis(bromomethyl)-2,8-dimethylpyrimido[4,5-*g*]quinazoline-4,9-(3*H*,8*H*)-dione (9b). To a solution of 48 mg (0.14 mmol) of **8b** in 10 mL of dimethyl formamide was added 2 mL of 48% aqueous hydrobromic acid. The reaction mixture was stirred for 24 h, and the crystallized product was filtered off and dried: 21 mg (35%) yield; TLC (dichloromethane/methanol [95:5]), R_f = 0.53; IR (KBr pellet) 3455, 1690, 1595, 1474, 1350, 1101, 795 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.60 (2H, s, H(5) and H(10) aromatic), 4.51 (4H, s, C(2) andC(7) bromomethyls), 3.78 (6H, s, N(3) and N(8) methyls); mass spectrum (EI mode), m/z 426 (M^+, $^{79}Br^{79}Br$), 428 (M^+, $^{79}Br^{81}Br$), 430 (M^+, $^{81}Br^{81}Br$). Anal. ($C_{14}H_{12}Br_2N_4O_2$) H, N. Low carbon, 39.28% calculated versus 40.18% due to partial hydrolysis and loss of bromide.

2,7-Bis(bromomethyl)-3,8-bis(*n*-propyl)pyrimido[4,5-*g*]quinazoline-4,9-(3*H*,8*H*)-dione (9c). To a solution of 16 mg of **8c** in 3 mL of dimethylformamide was added 0.5 mL of 48% aqueous hydrobromic acid. The reaction mixture was stirred for 15 h, and the solvent was removed. The resulting solid was neutralized with saturated $NaHCO_3$ solution and extracted with 5 × 10 mL of CH_2Cl_2. Purification was carried out on a silica gel prep plate employing CH_2Cl_2/methanol (98/2) as eluent: 4 mg yield of product; mp >220 °C (dec); TLC (chloroform/methanol [9:1]), R_f = 0.70; ^{1}H NMR ($CDCl_3$) δ 8.59 (2H, s, C(5) and C(10) aromatic protons), 4.49 (4H, s, C(2) and C(7) bromomethyls), 4.16 (4H, q, J = 5.7 Hz, propylmethylenes adjacent to N), 1.81 (4H, sextet, J = 7.7 Hz, other propylmethylenes), 1.04 (6H, t, J = 7.5 Hz, propylmethyls); mass spectrum (EI mode), m/z 483(M^+, $^{79}Br^{79}Br$), 485 (M^+, $^{79}Br^{81}Br$), 487 (M^+, $^{81}Br^{81}Br$). Anal. ($C_{18}H_{20}Br_2N_4O_2$) C, H, N.

2,7-Bis(acetoxymethyl)-3,8-dimethylpyrimido[4,5-*g*]quinazoline-4,9-(3*H*,8*H*)-dione (10). To a stirred suspension of 100 mg (0.33 mmol) of **7b** in 15 mL of pyridine was added 100 μL of acetic anhydride. After the reaction mixture was stirred for 2 h at room temperature, the solvent was removed in vacuo and the solid residue was triturated with 40 mL of water for 15 min. The solid was filtered off and recrystallized from chloroform/hexane: 67 mg (52%) yield; mp 263−264 °C; TLC (chloroform/methanol [9:1]), R_f = 0.53; IR (KBr pellet) 1744, 1691, 1674, 1609, 1452, 1341, 1248, 1236, 1074, 794 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 8.31 (2H, s, C(5) and C(10) aromatic protons), 5.29 (4H, s, C(2) and C(7) acetoxymethyls), 3.56 (6H, s, N(3) and N(8) methyls), 2.21 (6H, s, acetylmethyls); mass spectrum (EI mode), m/z 386 (M^+), 343 ($M^+ - COCH_3$). Anal. ($C_{18}H_{18}N_4O_6$) C, H, N.

1, 5-Dicarboxy-2,4-bis(chloroacetamido)benzene (12). To a suspension of 50 mg (0.185 mmol) of **11**[14] in 125 mL of dry benzene was added 37 μL of chloroacetyl chloride and 63μL of pyridine. The reaction mixture was stirred for 14 h. The benzene was removed in vacuo to afford a white residue. This solid residue was washed with a 1.0 N hydrochloric acid solution and then rinsed with cold distilled water: 47 mg (72%) yield. Analytically pure product was prepared by dissolving 150 mg of product in 60 mL of hot acetone and then adding boiling water dropwise. Product was obtained as ivory needles: 135 mg (90%) yield; mp >265 °C (dec); TLC (2-propanol/water/ammonium hydroxide [7:2:1]), R_f = 0.53; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.85 and 8.67 (2H, 2s, aromatic), 4.50 (4H, s, chloromethyls); IR (KBr) 3408, 3161, 2958, 1697, 1593, 1531, 1394, 1329, 1232, 1122 cm^{-1}; mass spectrum (El mode), m/z 348 (M^+, $^{35}Cl^{35}Cl$), 349 ($M^+$$^{35}Cl^{37}Cl$), 350 ($M^+$, $^{37}Cl^{37}Cl$). Anal. ($C_{12}H_{10}Cl_2N_2O_6 \cdot H_2O$) C, H, N.

4,1-Benzo[1,3-*e*:6,5-*e*′]bisoxazepine-2,5,7,10-(1*H*, 3*H*, 9*H*, 11*H*)-tetrone (13). To 205 mg (0.59 mmol) of **12** in 250 mL of dry dimethylformamide at 75 °C was added 0.65 mL (4.71

mmol) of triethylamine. The reaction mixture was stirred for 22 h at 85 °C. The dimethylformamide was removed in vacuo to afford a brown solid residue. The residue was suspended in 20 mL of distilled water and extracted 7× with 15 mL portions of ethyl acetate. The ethyl acetate extracts were dried over sodium sulfate and then concentrated to 5 mL. The mixture was chilled for 2 h, and the crystallized solids were filtered off. The crude yield after filtration and rinsing with hexane was 100 mg (62%). Analytically pure material was prepared by dissolving a small quantity of product in acetone. To the acetone was added 0.5 g of TLC standard grade silica gel (average particle size of 2.25 μm). The mixture was filtered, and the silica gel was rinsed with acetone. The acetone was evaporated to dryness, affording a pale-yellow powder: mp >270 °C (dec); TLC (chloroform/methanol [9:1]), $R_f = 0.21$; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 11.25 (2H, s, 1- and 11-amino protons), 8.28 and 6.63 (2H, 2s, aromatic), 4.76 (4H, s, methylenes); IR (KBr) 3275, 3124, 2972, 1699, 1610, 1411, 1363, 1221, 1134, 889 cm^{-1}; mass spectrum (El mode), *m*/*z* 276 (M^+). Anal. ($C_{12}H_8N_2O_6$) C, H, N.

4,6-Bis(cyanoacetamido)-1,3-bis(methylcarbamyl)benzene (14). A 50 mL round-bottom flask containing 30 mg (0.11 mmol) of **13** and 100 mg (2 mmol) of sodium cyanide was placed in a dry ice/2-propanol bath. To this was added 15 mL of a 21.6% methanolic methylamine solution. The reaction flask was stoppered and allowed to equilibrate to room temperature over a period of 20 min. The reaction mixture was stirred for 2 h at 40 °C. During this time, fibrous crystals of product formed. The crude yield after rinsing with cold methanol is variable: 10−16 mg (26−44%). Analytically pure product was obtained by recrystallization from dimethyl sulfoxide: mp >338 °C (dec); TLC (chloroform/methanol [9:1]), $R_f = 0.32$; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 8.99 (2H, br q, J = 6 Hz, 1- and 3-carbamyl protons), 8.95 and 7.91 (2H, 2s, aromatic [no assignments made]), 3.51 (4H, s, cyanomethylenes), 2.83 (6H, d, J = 6 Hz, 1- and 3-carbamylmethyls); IR (KBr) 3402, 3325, 1678, 1581, 1533, 1464, 1427, 1369, 1278, 1149 cm^{-1}; mass spectrum (El mode), *m*/*z* 356 (M^+). Anal. ($C_{16}H_{16}N_6O_4$) C, H, N.

4,6-Diamino-1,3-bis(methylcarbamyl)benzene (15). To 50.7 mg (0.150 mmol) of **14** was added 50 mL of a 2% aqueous sodium hydroxide solution. The mixture was stirred for 1 h at room temperature. The reaction mixture was extracted 8× with 120 mL portions of ethyl acetate. The ethyl acetate extracts were pooled, dried over Na_2SO_4, and evaporated to a 5 mL volume. Addition of hexane afforded product as a tan solid: 28 mg (52%) yield; mp >220 °C (dec); TLC (chloroform/methanol [9:1]), $R_f = 0.16$; ^{1}H NMR (dimethyl sulfoxide-d_6) 7.74 (2H, br q, J = 6 Hz, carbamyl protons), 7.68 and 5.79 (2H, 2s, aromatic [no assignments made]), 6.63 (4H, bs, 4- and 6-amino protons), 2.71 (6H, d, J = 6 Hz, 1- and 3-carbamylmethyls); IR (KBr) 3445, 3329, 2935, 1620, 1558, 1411, 1325, 1292, 1157, 923 cm^{-1}; mass spectrum (El mode), *m*/*z* 222 (M^+).

2,8-Bis(chloromethyl)-3,7-dimethylpyrimido[5,4-*g*]quinazoline-(3*H*,7*H*)-4,6-dione (16). 16 was prepared by the two steps described below.

To a suspension of 30 mg (0.1545 mmol) of **15** in 25 mL of dry benzene was added 27 μL (0.34 mmol) of chloroacetyl chloride and 27.5 μL (0.34 mmol) of pyridine. The reaction mixture was stirred at room temperature for 20 h under a dry atmosphere. The benzene was evaporated in vacuo to afford a solid white residue: 41 mg (71%) yield. Analytically pure 4,6-bis(chloroacetamido)-1,3-bis(methylcarbamyl)benzene was isolated from preparative TLC plates (chloroform/methanol [9:1]): mp >232 °C (dec); TLC (chloroform/methanol [9:1]), $R_f = 0.32$; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.61 and 8.13 (2H, 2s, aromatic [no assignments made]), 8.68 (2H, br q, J = 6 Hz, carbamyl protons), 4.43 (4H, s, chloromethyl), 2.82 (6H, d, J = 6 Hz, 1- and 3-carbamylmethyls); IR (KBr) 3402, 3323, 1680, 1585, 1467, 1346, 1278, 1128, 1036 cm^{-1}; mass spectrum (El mode), *m*/*z* 374 (M^+, $^{35}Cl^{35}Cl$), 375, (M^+, $^{35}Cl^{37}Cl$), 376 (M^+, $^{37}Cl^{37}Cl$).

To a solution of 11 mg (0.026 mmol) of the product obtained above in 5 mL of acetic acid was added 53 μL (0.805 mmol) of concentrated sulfuric acid. The mixture was refluxed for 1 h. The acetic acid was removed in vacuo to afford a viscous pale-yellow oil. The oily mixture was neutralized with an aqueous $NaHCO_3$ solution, resulting in the formation of white crystals. The crude yield after filtration and rinsing with cold distilled water was 54 mg (60%). Analytically pure product was obtained by preparative TLC (chloroform/methanol [9:1]): mp > 250 °C (dec); TLC (chloroform/methanol [9:1]), $R_f = 0.57$; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 8.90 and 7.82 (2H, 2s, aromatic [no assignments made]), 4.94 (4H, s, chloromethyls), 3.65 (6H, s, *N*-methyls); IR (KBr) 3047, 2995, 1687, 1589, 1423, 1346, 1263, 1120, 1016, 810 cm^{-1}; mass spectrum (El mode), *m*/*z* 338 (m^+, $^{35}Cl^{35}Cl$), 339 (M^+, $^{35}Cl^{37}Cl$), 340 (M^+, $^{37}Cl^{37}Cl$). Anal. ($C_{14}H_{12}Cl_2N_4O_2 \cdot 0.75H_2O$) C, H, N.

1,3-Dicarbamyl-2,5-dimethoxybenzene (18). To a solution consisting of 50 mL of ethanol and 1.0 g (5.4 mmol) of **17** that had been preheated to 40 °C were added 20 mL of 1 N sodium hydroxide and 20 mL of 10% hydrogen perioxide. After the reaction mixture of 40 °C was stirred for 30 min, the solvent was evaporated in vacuo and the residue was extracted with ethyl acetate. The ethyl acetate extracts were dried over Na_2SO_4 and evaporated to ∼5 mL. Addition of excess hexane afforded the crystalline product: 765 mg (64%) yield; mp 175−177 °C; TLC (ethyl acetate/methanol [9:1]), $R_f = 0.19$; IR (KBr) 3463, 3367, 3195, 1680, 1659, 1599, 1467, 1437, 1419, 1051 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.73 and 7.60 (4H, 2br s, 1- and 3-carbamyl protons [no assignments made]), 7.19 (2H, s, 4- and 6-aromatic protons), 3.78 and 3.75 (6H, 2s, 2- and 5-methoxys [no assignments made]); mass spectrum (EI mode), *m*/*z* 224 (M^+).

2,5-Dimethoxyisophthalic Acid Methyl Ester (19). 19 was prepared by the following two-step procedure. To a mixture consisting of 3 mL water and 1.5 mL of sulfuric acid was added 150 mg (0.67 mmol) of **18**, and the resulting solution was heated at 120 °C for 5 h. After cooling to room temperature, the solution was diluted with water (to ∼20 mL total volume) and then extracted with ethyl acetate. The ethyl acetate extracts were combined, dried over Na_2SO_4, and then evaporated to ∼10 mL. Addition of hexane gave the dicarboxylic acid: 140 mg (92%) yield; TLC (isopropanol−water/ammonium hydroxide [7:12]), $R_f = 0.38$; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.32 (2H, s, 4- and 6-aromatics), 3.79 and 3.75 (6H, 2s, 2- and 5-methoxys [no assignments made]).

To 5 mL of thionyl chloride was added 103 mg (0.45 mmol) of the dicarboxylic acid, and the resulting solution was refluxed for 30 min. After the solution was cooled to room temperature, the solvent was evaporated in vacuo. To the oily residue was added a solution consisting of l30 μL of triethylamine in 5 mL of dry methanol. The resulting mixture was stirred for 5 min, and then the solvents were evaporated to afford a residue that was then combined with ∼15 mL of water. The aqueous solution was extracted 3× with 20 mL portions of ethyl acetate. The extracts were combined, washed with 5% sodium carbonate, and then dried with Na_2SO_4. The desired diester was obtained as a thick oil upon evaporation of the solvent: 101 mg (88%) yield; TLC (ethyl acetate), $R_f = 0.62$; ^{1}H NMR ($CDCl_3$) δ 7.45 (2H, s, 3- and 5-aromatic protons), 3.93, 3.88, and 3.84 (12H, 3s, *O*-methyls [no assignments made]); mass spectrum (EI mode), *m*/*z* 254 (M^+).

1,3-Bis(methoxycarbonyl)-2,5-dimethoxy-4,6-dinitrobenzene (20). To a solution of 1.0 g (0.0039 mol) of **19** in 35 mL of acetic anhydride cooled to 5 °C was added 30 mL of 90% fuming nitric acid over a period of 2 h. The temperature of the reaction mixture was kept between 0 and 10 °C for 1 h after the addition of fuming nitric acid, and the mixture was then poured over 300 g of crushed ice followed by neutralization with $NaHCO_3$. The aqueous mixture was extracted 5× with 150 mL portions of ethyl acetate, and the organic layers were combined. The ethyl acetate was dried over Na_2SO_4, filtered, and evaporated to afford a red-brown viscous oil: 900 mg (67%) crude yield. Analytically pure product was obtained from column chromatography on silica, eluting with chloroform/ethanol (15:5): 800 mg (60%) yield; mp 65−66 °C; TLC (ethyl acetate), $R_f = 0.73$; ^{1}H NMR ($CDCl_3$) δ 4.01 and 3.97 (6H, 2s,

methoxy, no assignments made), 3.94 (6H, s, methyl esters); IR (KBr) 3011, 2960, 1747, 1547, 1438, 1336, 1228, 1030, 997, 868, 788 cm^{-1}; mass spectrum (EI mode), *m/z* 344 (M^+). Anal. ($C_{12}H_{12}N_2O_{10}$) C, H, N.

4,6-Diamino-1,3-bis(methoxycarbonyl)-2,5-dimethoxy benzene (21). A solution of 1.13 g (3.285 mmol) of **20** in 100 mL of hot ethanol was treated with a solution of 4.58 g (36.3 mmol) of sodium hydrosulfite in 40 mL of pH 7 buffer. The mixture was refluxed for 1 h. The ethanol was removed in vacuo, causing a white solid to precipitate.The aqueous mixture was extracted 8× with 100 mL portions of ethyl acetate. The ethyl acetate was dried over Na_2SO_4 and then evaporated to afford a pale-yellow paste. The crude yield was 0.530 g (57%). Pure product was obtained by column chromatography on silica gel by eluting with chloroform/methanol (9:1).

To prepare the hydrochloride salt, 130 mg of product were dissolved in 3 mL of ethanol to which was added 1 mL of concentrated HCl. The addition of ethyl acetate to this mixture and chilling afforded the crystalline product as an ivory solid. Physical data of the base form are the following: TLC (ethyl acetate), R_f = 0.70; ^{1}H NMR ($CDCl_3$) δ 3.88 (6H, s, methyl esters), 3.73 and 3.70 (6H, 2s, methoxy [no assignments made]); IR (KBr of HCI sale) 3472, 3338, 2953, 2804, 2592, 1685, 1591, 1437, 1338, 1255, 1012 cm^{-1}; mass spectrum (EI mode), *m/z* 284 (M^+).

4,6-Diamino-2,5-dimethoxy-1,3-bis(*N*-methylcarbamyl-)benzene Hydrochloride (22). A 500 mL round-bottom flask containing 0.500 g (1.76 mmol) of **21** and 2.5 g of NaCN was placed in a 2-propanol/dry ice bath. To this was added 50 mL of a 48% methanolic methylamine solution, and the flask was then sealed tightly and equilibrated to room temperature. The mixture was heated at 40 °C for 8 days. The flask was chilled in an 2-propanol/dry ice bath and vented, and the mixture was evaporated to dryness under reduced pressure to afford a tan paste. The paste was dissolved in 15 mL of distilled water and extracted 7× with 25 mL portions of ethyl acetate. The organic layers were combined, dried over Na_2SO_4, and evaporated to afford a light-brown paste. Pure product was obtained from column chromatography on silica gel by eluting first with 100% ethyl acetate to remove any starting material and monomethylcarbamyl derivative and then with 8% methanol in chloroform to remove the product: 330 mg (61%) yield.

To prepare the hydrochloric salt, an amount of 330 mg of product was dissolved in 3 mL of ethanol, to which was added 2 mL of concentrated HCl. The addition of ethyl acetate to this mixture and chilling afforded the crystalline product as an ivory solid: 230 mg (58%) yield; TLC (*n*-butanol/acetic acid/water [5:2:3]), R_f = 0.58; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.97 (2H, broad quartet, 1- and 3-carbamyl protons), 5.78 (4H, bs, 4- and 6-amino protons), 3.58 (6H, bs, methoxys [no assignments made]), 2.76 (6H, d, J = 6 Hz, 1- and 3-carbamylmethyls); IR (KBr) 3472, 3373, 3327, 2943, 2692, 1633, 1543, 1462, 1404, 1302, 1276, 1128, 995 cm^{-1}; mass spectrum (EI mode), *m/z* 282 (M^+). Anal. ($C_{12}H_{18}N_4O_4 \cdot HCl \cdot 0.5H_2O$) C, H, N.

4,6-Bis(chloroacetamido)-1,3-bis(methylcarbamyl)-2,5-dimethoxybenzene (23). To a suspension of 42 mg (0.12 mmol) of **22** in 25 mL of dry benzene was added 23 μL (0.29 mmol) of chloroacetyl chloride and 43 μL (0.53 mmol) of pyridine. The reaction mixture was stirred for 20 h at room temperature under a dry atmosphere. During this time, fibrous crystals of product formed. The benzene was evaporated in vacuo to afford a white solid, which was suspended in 10 mL of distilled water. The solids where filtered off, rinsed with cold water, and dried: 37 mg (74%) yield. Pure product was obtained as needles by recrystallization from hot ethyl acetate: mp >290 °C (dec); TLC (chloroform/methanol [9:1]), R_f = 0.49; ^{1}H NMR ($CDCl_3$) δ 9.73 (2H, s, 4- and 6-acetamido NH), 7.93 (2H, broad quartet, J = 6 Hz, 1- and 3-carbamyl protons), 4.21 (4H, s, chloromethyls), 3.70 and 3.59 (6H, 2s, methoxys [no assignments made]), 270 (6H, d, J = 6 Hz, 1- and 3-carbamylmethyls); IR (KBr) 3271, 3014, 2951, 1676, 1527, 1415, 1327, 1217, 1049, 1003 cm^{-1}; mass spectrum (El mode), *m/z* 434 (M^+, $^{35}Cl^{35}Cl$), 436 (M^+, $^{35}Cl^{37}Cl$).

2,8-Bis(chloromethyl)-5,10-dimethoxy-3,7-dimethylpyrimido[5,4-g]quinazoline-4,6-(3*H*,7*H*)-dione (24). To a solution of 76 mg (0.175 mmol) of **23** in 40 mL of acetic acid was added 29 μL (5.4 mmol) of concentrated sulfuric acid. The mixture was refluxed for 1 h, and the solvent was evaporated to afford a viscous yellow oil The oily mixture was neutralized to pH 7 with aqueous $NaHCO_3$, resulting in formation of crystalline product. The crude yield after rinsing with cold distilled water was 56 mg (80%). Analytically, pure product was obtained by recrystallization from hot ethyl acetate: mp >256 °C (dec); TLC (chloroform/methanol [9:1]), R_f = 0.57; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 4.91 (4H, s, chloromethyls), 4.01 and 3.85 (6H, 2s, methoxys [no assignments made]), 3.56 (6H, s, 3- and 7-*N*-methyls); IR (KBr) 2982, 2933, 1695, 1602, 1574, 1421, 1288, 1053, 914, 827 cm^{-1}; mass spectrum (EI mode), *m/z* 398 (M^+, $^{35}Cl^{37}Cl$), 400, (M^+, $^{35}Cl^{37}Cl$). Anal. ($C_{16}H_{16}Cl_2N_4O_4$) C, H, N.

3,6-(Dicarboxylic acid)-1,4-dimethoxy-2,5-dinitrobenzene (26). To 400 mg of 3,6-(dicarboxylic acid)-1,4-dimethoxy-2,5-dinitrobenzene dimethyl ester[1] in a solution of 20 mL of methanol at room temperature was added a solution of 20 mL of 25% KOH (aq). This mixture was stirred at room temperature for 30 min. The solution was then acidified to pH 3 with concentrated HCl. The acidified solution was extracted 6× with 30 mL of chloroform. The extracts were dried over sodium sulfate and filtered and the solvent was removed under reduced pressure. Product was recrystallized from chloroform/hexane: 250 mg (68%) yield; mp 148−151 °C (dec); TLC (2-propanol/water/ammonia [7:1:2]), R_f = 0.36; IR (KBr pellet) 3404, 2997, 2874, 1720, 1556, 1444, 1386, 1354 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.876 (s, 6 H, methoxys); mass spectrum (EI), *m/z* 316 (M^+), 272 ($M^+ - CO_2$), 228 ($M^+ - 2CO_2$). Anal. ($C_{10}H_8N_2O_{10}$) C, H, N.

3,6-Bis(2-methoxyethylcarbamyl)-1,4-dimethoxy-2,5-dinitrobenzene (27a). A mixture of 500 mg (1.6 mmol) of **26** in 10 mL of thionyl chloride was refluxed until all solids were in solution. Excess thionyl chloride was removed by distillation to leave a residue that was dried under reduced pressure on a high vacuum pump. To the dried residue was added 10 mL of dry dichloromethane, and the solution was chilled to −70 °C in a dry ice/2-propanol bath. To this chilled solution was added 1.2 mL (14.14 mmol) of 2-methoxyethylamine. A white solid formed immediately, which was filtered, washed with water, and recrystallized in ethanol: 358 mg (52%) yield; mp 167−170 °C; TLC (*n*-butanol/water/acetic acid [5:3:2], R_f = 0.88; IR (KBr pellet) 3279, 3094, 2897, 1656, 1543, 1398, 1302, 1130, 1028 cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 9.136 (t, 2 H, J = 5.4 Hz, amide protons), 3.813 (s, 6 H, methoxy protons), 3.387 (m, 8 H, J = 3.9 Hz, methylene protons), 3.246 (s, 6 H, methoxy protons); mass spectrum (EI), *m/z* 430 (M^+). Anal. ($C_{16}H_{22}N_4O_{10}$) C, N, H.

3,6-Bis(3-methoxypropylcarbamyl)-1,4-dimethoxy-2,5-dinitrobenzene (27b). A solution of 500 mg (1.6 mmol) of **26** in 10 mL of thionyl chloride was refluxed until all solids were in solution. Excess thionyl chloride was removed by distillation to leave a residue that was dried under reduced pressure on a high vacuum pump. To the dried residue was added 10 mL of dry dichloromethane, and the solution was chilled to −70 °C in a dry ice/2-propanol bath. To this chilled solution was added 1.23 mL (12.06 mmol) of 3-methoxypropylamine. The white solid, which formed in seconds, was filtered, washed with water, and recrystallized in ethanol: 509 mg (70.2%) yield; mp 216−218 °C (dec); TLC (*n*-butanol/water/acetic acid [5:3:2], R_f = 0.85; IR (KBr pellet) 3277, 3092, 2987, 2941, 2877, 2831, 1653, 1547, 1469, 1369, 1300, 1222, 1126 cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 9.024 (t, 2 H, J = 5.5 Hz, amide protons), 3.810 (s, 6 H, methoxy protons), 3.352−3.215 (m, methylene and methoxy protons), 1.676 (m, 4 H, J = 6.6 Hz, methylene protons); mass spectrum (EI), *m/z* 458 (M^+). Anal. ($C_{18}H_{26}N_4O_{10}$) C, H, N.

2,7-Bis(chloromethyl)-5,10-dimethoxy-3,8-bis(2-methoxyethyl)pyrimido[4,5-*g*]quinazoline-4,9-dione (28a). 28a was synthesized utilizing the following two-step procedure. A mixture consisting of 100 mg (0.23 mmol) of **27a**, 60 mL of

methanol, and 20 mg of 5% Pd/charcoal catalyst was shaken at 50 psi of H_2 for 6 h. Upon completion, the reaction mixture was opened to the air and filtered through a thin pad of Celite. The solvent was removed, and the residue was dried under reduced pressure. In a dry atmosphere, 4 mL of dry benzene and 1 mL of dry pyridine were added followed by 40 μL (0.484 mmol) of chloroacetyl chloride. This mixture was stirred at room temperature for 9 h, after which the solids were filtered off, washed with water, and dried under reduced pressure. The dried solids were immediately dissolved in 2 mL of acetic acid. This solution was placed in a round-bottom flask and fitted with a condenser, and a catalytic amount of H_2SO_4 was added. The mixture was refluxed for 30 min, at which time the solvent was removed under reduced pressure. The flask with the residue was placed in an ice bath, chloroform was added, and the biphasic mixture was neutralized with aqueous sodium bicarbonate solution. The extracts were dried with sodium sulfate and filtered, and the solvent was removed under reduced pressure. The residue was placed on a silica gel flash column, and the product was eluted with a 1% solution of methanol in chloroform. The yellow product eluted first and was recrystallized in dichloromethane/hexane: 16.5 mg (14.7% overall) yield; mp 177−179 °C (dec); TLC (methanol/chloroform [1:9]), $R_f = 0.71$; IR (KBr pellet) 3464, 3001, 2941, 2903, 2827, 1680, 1604, 1450, 1367, 1267, 1190, 1097 cm $^{-1}$; ^{1}H NMR ($CDCl_3$) δ 4.880 (4H, s, methylene protons), 4.467 (4H, t, J = 3.9 Hz), 4.148 (6H, s, methoxys), 3.729 (4H, t, J = 4.5 Hz), 3.304 (6H, s, methoxys); mass spectrum (EI), m/z 486 (M^+, $^{35}Cl^{35}Cl$), 488 (M^+, $^{35}Cl^{37}Cl$), 490 (M^+, $^{37}Cl^{37}Cl$). Anal. ($C_{20}H_{24}Cl_2N_4O_6 \cdot 0.5H_2O$) C, H, N.

2,7-Bis(chloromethyl)-5,10-dimethoxy-3,8-bis(3-methoxypropyl)pyrimido[4,5-*g*]quinazoline-4,9-dione (28b). **28b** was synthesized utilizing the following two-step procedure. A mixture consisting of 100 mg (0.22 mmol) of **9**, 60 mL of methanol, and 20 mg of 5% Pd/charcoal catalyst was shaken at 50 psi of H_2 for 6 h. Upon completion, the reaction mixture was opened to the air and filtered through a thin pad of Celite. The solvent was removed, and the residue was dried under reduced pressure. In a dry atmosphere, 4 mL of dry benzene and 1 mL of dry pyridine was added followed by 40 μL (0.484 mmol) of chloroacetyl chloride. This mixture was stirred at room temperature for 9 h, after which the solids were filtered off, washed with water, and dried under reduced pressure. After drying, the solids were immediately dissolved in 2 mL of acetic acid. This solution was placed in a round-bottom flask and fitted with a condenser, and a catalytic amount of H_2SO_4 was added. The mixture was refluxed for 30 min, at which time the solvent was removed under reduced pressure. The flask with the residue was placed in an ice bath, chloroform was added, and the biphasic mixture was neutralized with aqueous sodium bicarbonate solution. The extracts were dried with sodium sulfate and filtered, and the solvent was removed under reduced pressure. The residue was placed on a silica gel flash column, and the product was eluted with a 1% solution of methanol in chloroform. The yellow product eluted first; it was recrystallized in dichloromethane/hexane: 14 mg (12% overall) yield; mp 120−123 °C; TLC (methanol/chloroform [1:9]), $R_f = 0.73$; IR (KBr pellet) 3460, 2995, 2938, 2896, 2800, 1650, 1596, 1424, 1330, 1240, 1140, 1050 cm $^{-1}$; ^{1}H NMR ($CDCl_3$) δ 4.745 (4H, s, methylene protons), 4.302 (4H, t, J = 5.8 Hz), 4.121 (6H, s, methoxys), 3.433 (4H, t, J = 5.4 Hz), 3.345 (6H, s, methoxys), 2.086 (4H, m, methylene); mass spectrum (EI), m/z 514 (M^+, $^{35}Cl^{35}Cl$), 516 (M^+, $^{35}Cl^{37}Cl$), 518 (M^+, $^{37}Cl^{37}Cl$). Anal. ($C_{22}H_{28}N_4O_6Cl_2$) C, H, N.

Topoisomerase II Inhibition Assays. The topoisomerse II relaxation reactions were carried out with 0.25 μg of pRYG supercoiled DNA (form I), 4 units of topoisomerase II in a total volume of 20 μL of 50 mM Tris buffer (pH 8.0) containing 120 mM of KCL, 10 mM of $MgCl_2$, 0.5 mM of dithiothreitol, and 0.5 mM of ATP in microcentrifuge tubes. Varying amounts of drugs were added to each reaction. The reactions were run at 37 °C for 45 min. The reactions were then stopped by the addition of 2 μL of 10% SDS to each tube, and 1 μL of proteinase K (1 mg/mL) was also added. The resulting mixtures were incubated for 15 more min at 37 °C. Each reaction mixture was extracted once with 20 μL of chloroform/isoamyl alcohol (24:1) and then combined with 2 μL of 10X loading dye (0.25% bromophenol blue and 50% aqueous glycerol). The resulting mixtures were then loaded onto a 1% agarose gel in 1X TAE buffer and run at 2 V/cm for 5−7 h. The gels either contained or not contained 0.5 μg/mL ethidium bromide. After running, gels without ethidium bromide were soaked in 1X TAE buffer containing 0.5 μg/mL of ethidium bromide for 30 min followed by destaining in 1X TAE buffer for 20 min.

In Vivo Evaluation. The B-16 melanoma in C57/bl mice syngraft model was employed to determine in vivo activity.[19] Each agent was evaluated at three doses: 2, 3, and 5 mg kg^{-1} day^{-1} on days 1, 5, and 9 after subcutaneous tumor implantation of 10^5 cells in the front flank on day 0. "Toxic" means that there was early lethality or ≥50% lethality prior to any deaths in the control group. The treated over control values (T/C) were measured at day 25 of the study. A T/C value less than 40% is considered active. The control was obtained with drug-free animals.

Acknowledgment. We thank the National Institutes of Health, National Science Foundation, and the Arizona Disease Control Research Commission for their generous support.

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JM020285L