Synthesis and Evaluation of 6-(Dibromomethyl)-5-nitropyrimidines as Potential Antitumor Agents

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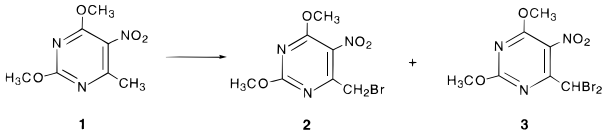
A series of 2,4,6-trisubstituted-5-nitropyrimidines have been prepared and evaluated for inhibition of proliferation of L1210 and H.Ep.2 cells *in vitro.* The most potent compound was 6-(dibromomethyl)-2-methoxy-4-morpholino-5-nitropyrimidine (**11**) (L1210, IC_{50} = 0.32 μM; H.Ep.2, IC_{50} = 1.6 μM). Of the 6-substituents incorporated, only $CHBr_2$, CH_2Br, and CHO were compatible with antiproliferative activity, while a wider variety of 4-substituents were tolerated. At concentrations near the IC_{50} for antiproliferative activity, a delayed resumption of cell proliferation in L1210 cultures indicated that the activity of the compounds was short-lived and suggested they might act by an alkylation mechanism.

Introduction

6-(Bromomethyl)-2,4-dimethoxy-5-nitropyrimidine (**2**) was prepared as a key intermediate in our study to prepare 2,4-dimethoxypyrrolo[3,2-*d*]pyrimidines.[1,2] The dibrominated derivative, 6-(dibromomethyl)-2,4-dimethoxy-5-nitropyrimidine (**3**), was obtained as a significant byproduct (31%) in the bromination reaction of 2,4-dimethoxy-6-methyl-5-nitropyrimidine (**1**). *In vitro* cytotoxicity screening of intermediates against L1210 and H.Ep.2 cells showed that the byproduct **3** exhibited more significant cytotoxicity than **2** against both of these cell lines. Following the lead presented by this finding, we now report the synthesis and cytotoxicity evaluation of a series of compounds related to 6-(dibromomethyl)-2,4-dimethoxy-5-nitropyrimidine (**3**).



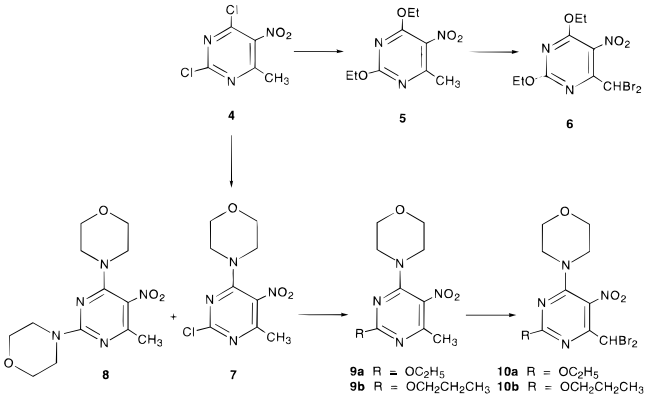
1 **2** **3**

Chemistry

The compounds described here were prepared by several methods. Following the procedure which was used to prepare **3**, 2,4-dichloro-6-methyl-5-nitropyrimidine (**4**)[3] was reacted with sodium ethoxide which afforded **5** as a light yellow solid (Scheme 1). Treatment of **5** with an excess of bromine in acetic acid at 50 °C gave 6-(dibromomethyl)-2,4-diethoxy-5-nitropyrimidine (**6**) in 70% yield. Under these forcing conditions no monobromomethyl derivative was detected by TLC. Reaction of **4** with morpholine afforded a mixture of 2-chloro-6-methyl-4-morpholino-5-nitropyrimidine (**7**)[4] and 2,4-dimorpholino-6-methyl-5-nitropyrimidine (**8**). Compound **7** was treated with sodium ethoxide and sodium *n*-propoxide to give the 2-ether derivatives **9a**,**b**, in yields of 49% and 72%, respectively. Bromination of **9a,b** furnished the dibromomethyl derivatives **10a**,**b**, respectively.

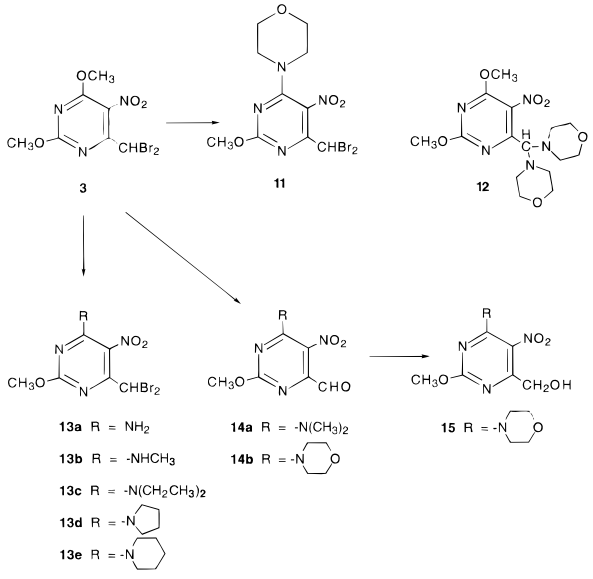
Reaction of the dibromomethyl derivative **3** with 2 equiv of anhydrous morpholine under conditions which were expected[5] to afford the 2,4-dimethoxy-6-*gem*-[(dimorpholino)methyl] compound **12** instead afforded 6-(dibromomethyl)-2-methoxy-4-morpholino-5-nitropyrimidine (**11**) (Scheme 2). The formation of **11** rather than **12** may be attributed to the activating effect of the

Scheme 1. Synthesis of Some 2-Ethoxy- and 2-Propoxy-5-nitro-6-(dibromomethyl)pyrimidines



4 **5** **6** **8** **7**
9a R = OC_2H_5 **10a** R = OC_2H_5
9b R = $OCH_2CH_2CH_3$ **10b** R = $OCH_2CH_2CH_3$

Scheme 2. Synthesis of Some 4-Substituted-2-methoxy-5-nitro-6-(dibromomethyl)pyrimidines and Related Compounds



3 **11** **12**
13a R = NH_2 **14a** R = $-N(CH_3)_2$ **15** R = -N O
13b R = $-NHCH_3$ **14b** R = -N O
13c R = $-N(CH_2CH_3)_2$
13d R = -N
13e R = -N

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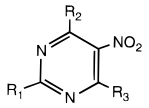
Table 1. Inhibition of Cell Proliferation *in Vitro* by 2,4,6-Trisubstituted-5-Nitropyrimidines



					L1210			H.Ep.2		
						IC50 (mM)			IC50 (mM)	
compd	R_1	R_2	R_3	screen conctn[a] (mM)	screen GR[b] (% of cont.)	growth rate[c]	survival[d]	screen GR[b] (% of cont.)	growth rate[c]	survival[d]
1	$-OCH_3$	$-OCH_3$	$-CH_3$	100	104	*e*	*e*	90	*e*	*e*
2	$-OCH_3$	$-OCH_3$	$-CH_2Br$	100	0	8.0[f]	25	0	ND	
3	$-OCH_3$	$-OCH_3$	$-CHBr_2$	100	0	1.9	ns[g]	0	2.5	2.7
6	$-OCH_2CH_3$	$-OCH_2CH_3$	$-CHBr_2$	100	0	7.2	ns	ND[h]		
10a	$-OCH_2CH_3$	-*N*-morpholino	$-CHBr_2$	100	13	3.6	ns	ND		
10b	$-O(CH_2)_2CH_3$	-*N*-morpholino	$-CHBr_2$	10	13	2.3	6.1	ND		
11	$-OCH_3$	-*N*-morpholino	$-CHBr_2$	100	0	0.32	0.56	0	1.6	2.8
13a	$-OCH_3$	$-NH_2$	$-CHBr_2$	100	0	2.0	ns	0	1.6	3.3
13b	$-OCH_3$	$-NHCH_3$	$-CHBr_2$	100	0	2.3	2.0	0	ND	
13c	$-OCH_3$	$-N(CH_2CH_3)_2$	$-CHBr_2$	100	0	5.8	5.6	0	ND	
13d	$-OCH_3$	-*N*-pyrrolidino	$-CHBr_2$	10	0	0.9	1.0	ND		
13e	$-OCH_3$	-*N*-piperidino	$-CHBr_2$	10	0	0.54	1.8	36	ND	
14a	$-OCH_3$	$-N(CH_3)_2$	-CHO	100	0	5.9	12	ND		
15	$-OCH_3$	-*N*-morpholino	$-CH_2OH$	100	100	*e*	*e*	100	*e*	*e*
16[2]	$-OCH_3$	$-OCH_3$	$-CH{=}P(C_6H_5)_3$	10	93	*e*	*e*	ND		
17[1]	$-OCH_3$	$-OCH_3$	$-CH{=}CH\text{-}N(CH_3)_2$	10	87	*e*	*e*	ND		
4[3]	-Cl	-Cl	$-CH_3$	100	104	*e*	*e*	ND		

[a] This concentration was the same for both cell lines, except for compounds **11** and **13a**. These compounds were evaluated at 100 μM in L1210 cells and at 10 μM in H.Ep.2 cells. [b] The initial growth rate of cells in the presence of the compound at the screen concentration. The growth rate is defined as the initial slope of the growth curve of treated cells, expressed as a percentage of the slope of the growth curve of untreated, control cells. [c] The concentration required to decrease the growth rate to 50% of control. [d] The concentration required to decrease the estimate of the minimum fraction of cells that survived treatment to 50%. This surviving fraction was estimated by back-extrapolating the growth curves of treated cells as described in the text. [e] Cell proliferation was not slowed at the screen concentration, which was the highest concentration tested. [f] Based on a single concentration−response determination. All others are the average of two determinations. [g] ns, no evidence of surviving cells at the growth inhibitory concentrations tested. [h] ND, not done.

5-nitro group toward nucleophilic reactions at the 4-position. Utilizing this reaction, a series of 4-amino-2-methoxy derivatives were prepared. Thus, reaction of **3** with ammonia, methylamine, diethylamine, pyrrolidine, and piperidine afforded the 4-amino derivatives **13a**−**e**.

Reaction of **3** with excess dimethylamine in glyme at room temperature followed by hydrolysis afforded 4-(dimethylamino)-6-formyl-2-methoxy-5-nitropyrimidine (**14a**) in 16% yield. Similarily, the reaction of **3** with neat morpholine at room temperature followed by hydrolysis afforded the intermediate 4-morpholino-6-formyl-2-methoxy-5-nitropyrimidine (**14b**) which was not isolated but was treated directly with sodium borohydride in methanol to afford the 6-hydroxymethyl derivative **15** in 78% yield. It is apparent that under these reaction conditions, the reaction proceeded to afford an intermediate 6-*gem*-diamino derivative which hydrolyzed during the hydrochloric acid wash to give a 6-formyl moiety.

Biology

The antitumor potential of the new compounds was evaluated by studying their ability to inhibit proliferation of L1210 murine leukemic cells and H.Ep.2 human epidermoid carcinoma cells *in vitro* (Table 1). 6-(Dibromomethyl)-2-methoxy-4-morpholino-5-nitropyrimidine (**11**) had the most potent antiproliferative activity, with IC_{50} = 0.32 μM for L1210 cells, and the 6-(dibromomethyl)-2,4-dimethoxy derivative **3** was only about 6-fold less potent. The 6-dibromomethyl substituent (R_3) appeared to play a crucial role in the antiproliferative mechanism of this series of compounds, since, for example, the potency of the 2,4-dimethoxy compounds (**1**−**3**, **16,** and **17**) was strongly influenced by their 6-substituents. Compound **3** with 6-dibromomethyl was the most potent, while the 6-bromomethyl derivative **2** was less potent, and the 6-methyl (**1**), 6-methylene triphenylphosphorane[2] (**16**), and 6-(dimethylamino)-ethylene[1] (**17**) derivatives were completely inactive. Compound **15** further exemplified this point. It differed from the most potent compound in the series (**11**) only by replacement of the 6-dibromomethyl with a 6-hydroxymethyl substituent, and it was completely devoid of activity. Therefore, most of the additional compounds in this series were synthesized with a 6-dibromomethyl substituent. The 4-morpholino substituent (R_2) also appeared to be important for the activity since replacing it with various linear or cyclic alkylamino or alkoxy substituents provided a series of compounds with decreasing potency: morpholino (**11**) > piperidino (**13e**) > pyrrolidino (**13d**) > amino (**13a**) = methylamino (**13b**) = methoxy (**3**) > diethylamino (**13c**). Lengthening the 2-alkoxy substituent (R_1) in **11** to ethoxy (**10a**) or propoxy (**10b**) caused about a 10-fold decrease in antiproliferative potency. When the 4-substituent (R_2) was also changed to ethoxy (**6**), the antiproliferative potency was decreased 2-fold further. Compound **14a**, with 4-$N(CH_3)_2$-6-CHO, was an exception to the requirement for a 6-$CHBr_2$ or 6-CH_2Br substituent. It retained antiproliferative activity, though it was 20-fold less potent than **11**. Although the corresponding 4-$N(CH_3)_2$-6-$CHBr_2$ analog was not available to provide a direct comparison, it must be concluded that the 6-CHO was compatible with antiproliferative activity. Compound

14b, with 4-morpholino-6-CHO was not available in sufficient quantity for evaluation.

The shapes of the growth curves for L1210 cells treated with many of these compounds suggested that their activity was short-lived. In cultures treated with **2**, **10b**, **11**, **13b**−**e**, or **14a** at concentrations near their IC_{50}'s, cell proliferation was initially slowed or stopped and later resumed at the control rate. This data would be explained if all the cells resumed proliferation after the compound decomposed over a time period that approximated the period of slowed cell proliferation.[6] In this case, the compound would be described as cytotstatic since it stopped the proliferation of the cells without killing them. Alternatively, these observations would also be explained if the compound killed a fraction of the cells during the initial few hours (i.e., a period that was very brief compared to the 3-day time course of the growth curve) during which the compound also lost its antiproliferative activity. Many alkylating agents exhibit this behavior in cell culture.[7] When the surviving fractions were derived from the growth curves assuming the latter model, the IC_{50}'s for survival in Table 1 were obtained. Of course, these values represent the minimum surviving fractions of the cells in the treated cultures. If the compounds were cytostatic, as in the first model, the actual surviving fraction could be as high as 100%. While it is speculative which of these models applies to the compounds in the present study, the well-known reactivity of alkyl halides suggested that the 6-dibromomethyl and 6-bromomethyl moieties might confer alkylating activity in this series of compounds. The activity of the 6-CHO derivative **14a** is assumed to be mediated by a different mechanism due to the chemical difference between the aldehyde and (di)halomethyl substituents. An additional note regarding the possible mechanism of action of compound **11** is that 10 μM dThd did not ameliorate its antiproliferative effect in L1210 cell cultures. Thus, this compound appeared not to act by inhibiting conversion of UMP to TMP. The possibility that **11** may interfere with *de novo* pyrimidine synthesis has not been tested.

Compounds **11** and **13a** were selected for *in vivo* antitumor evaluation to determine whether the approximately 10-fold difference in antiproliferative activity *in vitro* would be reflected *in vivo*. The compounds were injected once daily on days 1−5 after inoculation of the mice with P388 leukemic cells.[8] At doses of 200 or 100 mg/kg/injection, compound **11** shortened the life span of the mice, in comparison to untreated control leukemia-bearing mice (at 200 mg/kg, mice died before day 5; T/C%[8] = 87 at 100 mg/kg). At 50 or 25 mg/kg, no effect on life span was observed (T/C% = 101 and 109, respectively). Compound **13a** was even more toxic. Doses of 200−25 mg/kg/injection shortened the life span (at 200−50 mg/kg mice died before day 5; T/C% = 91 at 25 mg/kg), while 12.5 or 6.25 mg/kg had no effect (T/C% = 100 and 104, respectively). Thus, the antileukemic activity observed *in vitro* was overshadowed *in vivo* by toxicity, indicating that the antitumor potential of these compounds is likely to be limited.

Experimental Section

General Methods. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Silica gel 60 (230−400 mesh) was used for chromatography. Thin layer chromatography (TLC) was performed on prescored SilicAR 7GF plates. Detection of components was by ultraviolet light (254 nm). Evaporations were carried out under reduced pressure (water aspirator) with the bath temperature > 50 °C. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a 270-MHz spectrometer. Analytical samples were dried *in vacuo* at 78 °C in the presence of phosphorus pentoxide for at least 12 h unless otherwise specified. Elemental analyses are within 0.4% of true values and were performed by MHW, Phoenix, AZ.

2,4-Diethoxy-6-methyl-5-nitropyrimidine (5). To a solution of sodium ethoxide (from 6.4 g (278 mmol) of sodium) in absolute ethanol (400 mL) was added 2,4-dichloro-6-methyl-5-nitropyrimidine (**4**) (26.0 g, 125 mmol) in ethanol (100 mL), and the reaction mixture was stirred at ambient temperature for 3 h. Neutralization of the reaction mixture to pH = 7 with concentrated HCl gave a yellow mixture which was filtered. The filtrate was evaporated to afford a solid. Purification was achieved by column chromatography on silica gel eluting with 10−15% EtOAc in ligroin. The appropriate fractions were pooled and evaporated to give **5** (14.0 g, 49%) as a light yellow solid: ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 6 H, OCH_2CH_3), 2.47 (s, 3 H, 6-CH_3), 4.50 (dq, 4 H, OCH_2CH_3). Anal. ($C_9H_{13}N_3O_4$) C, H, N.

6-(Dibromomethyl)-2,4-diethoxy-5-nitropyrimidine (6). Compound **5** (14.6 g, 64.3 mmol) and sodium acetate (10.7 g, 130 mmol) were dissolved in glacial acetic acid (500 mL). The solution was warmed to 50 °C, and a solution of bromine (12.5 g, 79 mmol) in acetic acid (10 mL) was added. After 30 min, an additional similar portion of bromine was added, and the solution was warmed to 90 °C. After 3.5 h, another portion of bromine was added and the reaction mixture was heated to reflux. After 1 h, the reaction mixture was cooled and allowed to stand at room temperature for 13 h. Evaporation of the reaction mixture gave a yellow solid, which was suspended in EtOAc (200 mL). This mixture was decanted from the excess solid, extracted with saturated $NaHCO_3$, brine, and water, and then dried over $MgSO_4$. Filtration followed by evaporation gave **6** (17.3, 70%) as a nearly pure, viscous yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.50 (dt, 6 H, OCH_2CH_3), 4.55 (q, 4 H, OCH_2-CH_3), 6.80 (s, 1 H, 6-$CHBr_2$). An analytical sample was prepared by low pressure liquid chromatography eluting with 10% EtOAc in cyclohexane. Anal. ($C_9H_{11}Br_2N_3O_4$) C, H, N.

2-Chloro-6-methyl-4-morpholino-5-nitropyrimidine (7). Compound **4** (7.3 g, 35.1 mmol) was dissolved in Et_2O (150 mL), and the solution was cooled to −5 to −10 °C. A solution of morpholine (6.41 g, 73.7 mmol) in Et_2O (200 mL) was added dropwise over 1.5 h. The resulting mixture was filtered, and the solid (morpholine hydrochloride) was washed with Et_2O. Silica gel (10 g) was added, and the solvent was evaporated to afford a yellow powder which was added to the top of a silica gel column (2.5 × 20 cm). Elution with EtOAc/ligroin (1:1) gave two main yellow bands. Pooling the fractions which contained the faster running band and evaporation afforded the 4-morpholinopyrimidine derivative **7** (3.3 g, 36%) as a yellow solid: mp 125−126 °C; ^{1}H NMR ($CDCl_3$) δ 2.47 (s, 3 H, CH_3), 3.44−3.90 (m, 8 H, CH_2). Anal. ($C_9H_{11}ClN_4O_3$) C, H, N.

Evaporation of the fractions containing the slower running yellow band gave 2,4-dimorpholino-6-methyl-5-nitropyrimidine (**8**) (6% yield): mp 158−159 °C; ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3 H, CH_3), 3.00−4.00 (m, 16 H, CH_2). Anal. ($C_{13}H_{19}N_5O_4$) C, H, N.

2-Ethoxy-6-methyl-4-morpholino-5-nitropyrimidine (9a). Sodium (0.20 g, 8.7 mmol) was dissolved in absolute ethanol (150 mL), and the solution was cooled to room temperature. A solution of **7** (2.25 g, 8.7 mmol) in ethanol (75 mL) and ether (25 mL) was added in one portion. The addition of this yellow solution resulted in a deep red-orange reaction mixture. The reaction mixture was stirred at room temperature for 3 h. The precipitated solid (NaCl) was removed by filtration and washed with ether. Evaporation of the filtrate produced a dark oil that was submitted to chromatography on silica gel (2.5 × 10 cm) eluting with EtOAc to give pure **9a** (1.14 g, 49%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 1.32 (t, 3

H, CH_2CH_3), 2.42 (s, 3 H, 6-CH_3), 3.35−3.85 (m, 8 H, CH_2), 4.37 (q, 2 H, OCH_2CH_3). Anal. ($C_{11}H_{16}N_4O_4$) C, H, N.

2-(1-Propoxy)-4-morpholino-6-methyl-5-nitropyrimidine (9b). Freshly cut sodium (0.30 g, 13.0 mmol) was dissolved in dry 1-propanol. The chloropyrimidine **7** (3.37 g, 13.0 mmol) was dissolved in warm 1-propanol (200 mL). The two solutions were combined and stirred at room temperature for 48 h. The reaction mixture was treated with decolorizing carbon and filtered. Silica gel (10 g) was added to the filtrate, and the slurry was evaporated to a powder which was placed on a column of silica gel (100 g). Elution with EtOAc afforded **9b** as a yellow syrup (2.65 g, 72%): ^{1}H NMR ($CDCl_3$) δ 1.04 (t, 3 H, $OCH_2CH_2CH_3$), 1.90 (m, 2 H, $OCH_2CH_2CH_3$), 2.47 (s, 3 H, $ArCH_3$), 3.62 (m, 8 H, morpholino), 4.29 (t, 2 H, OCH_2-CH_2CH_3). Anal. ($C_{12}H_{18}N_4O_4$) C, H, N.

6-(Dibromomethyl)-2-ethoxy-4-morpholino-5-nitropyrimidine (10a). The ethoxypyrimidine **9a** (5.10 g, 19.0 mmol) and anhydrous NaOAc (4.1 g, 50 mmol) were dissolved in glacial acetic acid (200 mL). This solution was heated to reflux, during which time a solution of bromine (Br_2 (4 mL) in HOAc (25 mL)) was slowly added. The cloudy, red reaction mixture was stirred at reflux for 2 h and then allowed to stir at room temperature for 48 h. Evaporation gave an orange solid which was digested in boiling EtOAc. This solution was washed with $NaHCO_3$ and brine and then dried ($MgSO_4$). Silica gel was added, and the slurry was evaporated to a powder which was chromatographed eluting with EtOAc to give the (dibromomethyl)pyrimidine **10a** as the fastest running component of the mixture. Final purification was achieved using lobar chromatography eluting with hexane/EtOAc (2:1) giving **10a** as a viscous yellow oil (1.84 g, 23%): ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3 H, CH_3), 3.60 (m, 8 H, morpholino), 4.46 (q, 2 H, OCH_2), 6.97 (s, 1 H, $CHBr_2$). Anal. ($C_{11}H_{14}Br_2N_4O_4$) C, H, N.

6-(Dibromomethyl)-2-(1-propoxy)-4-morpholino-5-nitropyrimidine (10b). Propoxypyrimidine **9b** (2.65 g, 9.4 mmol) and anhydrous NaOAc (2.0 g, 24.4 mmol) were dissolved in glacial HOAc (150 mL). This solution was heated to reflux, during which time a solution of bromine (2 mL) in HOAc (15 mL) was slowly added. After refluxing for 2 h, the reaction mixture was allowed to stir at room temperature for 48 h. Evaporation gave a residue which was digested in boiling EtOAc (100 mL). This EtOAc solution was decanted from the insoluble residue, washed with $NaHCO_3$ and brine, and dried ($MgSO_4$). Silica gel was added, and the slurry was evaporated to a powder which was chromatographed eluting with EtOAc. Final purification was achieved with lobar chromatography eluting with hexane/EtOAc (2:1) to give **10b** as a viscous yellow oil (1.48 g, 36%): ^{1}H NMR ($CDCl_3$) δ 1.00 (t, 3 H, $O(CH_2)_2$-CH_3), 1.75 (m, 2 H, $O(CH_2)_2CH_3$), 3.60 (m, 8 H, morpholino), 4.40 (t, 2 H, $OCH_2CH_2CH_3$), 7.00 (s, 1 H, $CHBr_2$). Anal. ($C_{12}H_{16}Br_2N_4O_4$) C, H, N.

4-Amino-6-(dibromomethyl)-2-methoxy-5-nitropyrimidine (13a). To a solution of **3** (1.06 g, 3.0 mmol) in glyme (5 mL) was added saturated methanolic ammonia (saturated at 20 °C) (0.5 mL). The reaction vessel was sealed, and the mixture was stirred at ambient temperature for 10 h. The excess ammonia and solvent were evaporated *in vacuo*, and the resulting yellow solid was crystallized from methanol: yield 0.66 g (67%); mp 165−166 °C; ^{1}H NMR ($CDCl_3$) δ 4.05 (s, 3 H, OCH_3), 7.48 (s, 1 H, CH), 8.08 (bs, 2, NH_2). Anal. (C_6H_6-$Br_2N_4O_3$) C, H, N.

6-(Dibromomethyl)-4-(methylamino)-2-methoxy-5-nitropyrimidine (13b). To a solution of **3** (2.12 g, 6 mmol) in glyme (10 mL) was added saturated (20 °C) methanolic methylamine (1 mL). The reaction vessel was sealed and the mixture stirred at ambient temperature for 14 h. The solvent was evaporated *in vacuo*, and the resulting residue was dissolved in EtOAc (100 mL), washed once with 1 N HCl (30 mL), saturated aqueous $NaHCO_3$ (2 × 30 mL), and saturated aqueous NaCl (2 × 20 mL), and dried over $MgSO_4$. The solvent was evaporated to afford a solid which was crystallized from ethanol to give **13b**: yield 1.63 g (77%); mp 92.5−93.5 °C; ^{1}H NMR ($CDCl_3$) δ 3.1−3.25 (d, 3 H, $NHCH_3$), 4.10 (s, 3 H, OCH_3), 7.50 (s, 1 H, $CHBr_2$), 8.22−8.9 (bs, 1 H, NH). Anal. (C_7H_8-$Br_2N_4O_3$) C, H, N.

6-(Dibromomethyl)-4-(diethylamino)-2-methoxy-5-nitropyrimidine (13c). To a solution of **3** (1.8 g, 5 mmol) in glyme (10 mL) was added a 10% solution of methanolic dimethylamine (0.6 mL). The reaction vessel was sealed and the reaction stirred at ambient temperature for 14 h. The reaction mixture was evaporated to dryness, and the resulting solid was dissolved in EtOAc (10 mL), washed with 1 N HCl (30 mL), saturated $NaHCO_3$ (2 × 30 mL), and saturated NaCl (2 × 30 mL), and then dried over $MgSO_4$. The solvent was evaporated, and the resulting residue of **13c** was crystallized from methanol: yield 0.8 g (40%); mp 91.5−92.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3 H, CH_3), 3.66 (q, 2 H, CH_2), 4.03 (s, 3 H, OCH_3), 7.13 (s, 1 H, CH). Anal. ($C_{10}H_{14}Br_2N_4O_3$) C, H, N.

6-(Dibromomethyl)-2-methoxy-5-nitro-4-pyrrolidinopyrimidine (13d). To a solution of **3** (1.79 g, 5 mmol) in glyme (10 mL) was added pyrrolidine (0.5 mL in 5 mL methanol). The reaction mixture was allowed to stir at room temperature for 14 h. The solvent was evaporated and the brown residue dissolved in EtOAc (100 mL), washed with 1 N HCl (35 mL), saturated $NaHCO_3$ (2 × 35 mL), and saturated aqueous NaCl (2 × 35 mL), and dried over $MgSO_4$. The solvent was evaporated and the resulting residue was crystallized from methanol to give **13d**: yield 0.49 g (25%); mp 144−145 °C; UV (λ_{max}) ($\epsilon \times 10^3$) MeOH λ 245 (4210), 325 (1600), 350 (1651); ^{1}H NMR ($CDCl_3$) δ 1.86−2.22 (m, 4 H, -CH_2), 3.85 (m, 4 H, CH_2), 4.06 (s, 3 H, OCH_3), 7.10 (s, 1 H, CH). Anal. ($C_{10}H_{12}$-$Br_2N_4O_3$) C, H, N.

6-(Dibromomethyl)-2-methoxy-4-piperidino-5-nitropyrimidine (13e). To a solution of **3** (1.8 g, 5 mmol) in glyme (10 mL) was added a 10% solution of methanolic piperidine (0.6 mL of piperidine in 5.8 mL of methanol). The vessel was sealed, and the reaction mixture was stirred at ambient temperature for 14 h. The reaction mixture was evaporated, and the resulting residue was taken up in EtOAc (100 mL), washed with 1 N HCl (1 × 40 mL), saturated $NaHCO_3$ (2 × 30 mL), and saturated NaCl (1 × 30 mL), and dried over $MgSO_4$. The solvent was evaporated, and the resulting residue was crystallized from methanol to give **13e**: yield 0.78 g (38%); mp 140−141 °C; ^{1}H NMR ($CDCl_3$) δ 1.70 (m, 6 H, $(CH_2)_3$), 4.30 (m, 7 H, $(CH_2)_2$, OCH_3), 7.12 (s, 1 H, CH). Anal. ($C_{11}H_{14}$-$Br_2N_4O_3$) C, H, N.

4-(Dimethylamino)-6-formyl-2-methoxy-5-nitropyrimidine (14a). To a solution of **3** (1.43 g, 4 mmol) in glyme (10 mL) was added dimethylamine (1 g). The reaction vessel was sealed and the mixture stirred at ambient temperature for 14 h. The reaction mixture was evaporated, and the resulting solid was taken up in EtOAc (100 mL) and washed with 1 N HCl (1 × 40 mL), saturated $NaHCO_3$ (2 × 30 mL), and saturated NaCl (1 × 25 mL). The organic layer was dried over $MgSO_4$. The solvent was evaporated, and the resulting residue was purified by flash chromatography eluting with CH_2Cl_2. The appropriate fractions were pooled and evaporated. The residue was crystallized from methanol−water: yield 0.21 g (16%); mp 130−131 °C; ^{1}H NMR (DMSO-d_6) δ 3.25 (s, 6 H, CH_3), 4.06 (s, 3 H, OCH_3), 10.05 (s, 1 H, CHO). Anal. ($C_8H_{10}N_4O_4$) C, H, N.

6-(Hydroxymethyl)-2-methoxy-4-morpholino-5-nitropyrimidine (15). A solution of **3** (1.28 g, 3.6 mmol) in morpholine (5 mL) was stirred at ambient temperature for 14 h. The mixture solidified into a yellow mass which was suspended in ether (50 mL) and the solid collected by filtration. The yellow powder was added to a well-stirred mixture of EtOAc (50 mL), 1 N HCl (50 mL), and ice (50 mL). After 30 min the organic layer was separated, washed with saturated $NaHCO_3$ (3 × 30 mL) and saturated NaCl (2 × 20 mL), and dried over $MgSO_4$. Removal of the solvent afforded 0.47 g of the intermediate formyl derivative **14b** (as shown by ^{1}H NMR). This material (0.13 g) without further purification was dissolved in methanol and treated with $NaBH_4$ (30 mg). After 1 h, the light yellow precipitate which formed during the course of the reaction was collected by filtration, washed with MeOH, and air-dried to furnish **15**: yield 0.102 g (78%); mp 185−186 °C; ^{1}H NMR (DMSO-d_6) δ 3.60 (m, 8 H, morpholino), 4.06 (s, 3 H, OCH_3), 4.52 (d, 2 H, CH_2), 4.8 (t, 1 H, OH). Anal. ($C_{10}H_{14}N_4O_5$) C, H, N.

Cell Culture. The *in vitro* cytotoxicity against L1210 cells was evaluated as described previously.[9] L1210 cells were grown in static suspension culture with Fischer's medium for leukemic cells of mice, and the growth rate over a 3-day period was determined in the presence of the indicated concentrations of the test compound, by counting the cells once each day. Growth rate is defined as the slope of the growth curve (plot of the log of the cell number against time) for a treated culture, as a percentage of the slope for the control culture. The average population-doubling time of control cells was 12 h. The IC_{50} is defined as the concentration required to decrease the growth rate to 50% of the control. The estimates of the fraction of cells surviving treatment with the compound were derived from the growth curves by extrapolating the exponential portion of the curve back to zero time and expressing this intercept as a percentage of the initial number of cells in the culture.

The *in vitro* cytotoxicity against H.Ep.2 human epidermoid carcinoma cells (ATCC CCL 23) was evaluated in monolayer cultures.[10] For growth rate determinations, 2×10^4 cells were placed in replicate 25 cm^2 flasks, in control medium. After 1 day of incubation the medium was changed to compound-containing or control medium. Then growth was monitored by harvesting and counting the cells in two flasks from each treatment group, on days 1, 2, and 4 after adding the compounds. The data were plotted and analyzed as described previously[10] and above for L1210 cells. The average control population-doubling time was 19 h.

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