Synthesis and cytotoxic activity of 5-(1-hydroxy-2-haloethyl)-, 5-oxiranyl- and (E)-5-(2-iodovinyl)-2,4-dichloro (or dimethoxy) pyrimidines

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Short communication

Synthesis and cytotoxic activity of 5-(1-hydroxy-2-haloethyl)-, 5-oxiranyl- and (*E*)-5-(2-iodovinyl)-2,4-dichloro (or dimethoxy) pyrimidines

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Summary — A series of 5-(1-hydroxy-2-haloethyl) **6**, **7**, **13**, **14a**, 5-oxiranyl **8**, **9** and (*E*)-5-(2-iodovinyl)-2,4-dichloro(or dimethoxy)pyrimidines **11**, **12** were synthesized for evaluation as cytotoxic agents. The nuclear C-2 and C-4 substituents were determinants of activity since the 2,4-dichloro compounds **6**, **8** and **11** were more potent (ED_{50} = 0.2–0.3 μg/ml) than the corresponding 2,4-dimethoxypyrimidine analogues **7**, **9** and **12** (ED_{50} = 4–28 μg/ml), relative to melphalan (ED_{50} = 0.15 μg/ml), in the *in vitro* L1210 screen. Within the 2,4-dichloro series of compounds **6**, **8** and **11**, the C-5 substituent was not a determinant of activity. In contrast, in the 2,4-dimethoxypyrimidine series, the C-5 substituents influenced activity significantly where the relative potency order was oxiranyl **9** > -CH(OH)CH_2I **7** > (E)-CH = CHI **12** > CH(OH)CHI_2 **13**, CH(OH)CHBr(I) **14a** and CH(Br)CHOH(I) **14b**. The most active compound (*E*)-5-(2-iodovinyl)-2,4-dichloropyrimidine **11** exhibited weak activity in the *in vivo* P388 screen (% T/C = 116 for a 10 mg/kg ip dose) relative to the reference drug 5-fluorouracil (% T/C = 135 for a 20 mg/kg dose).

Résumé — Synthèse et activité cytotoxique de 5-(1-hydroxy-2-haloéthyl)-, 5-oxiranyl- et (*E*)-5-(2-iodovinyl)-2,4-dichloro (ou diméthoxy)pyrimidines. *Une série de 5-(1-hydroxy-2-haloéthyl)* ***6***, ***7***, ***13***, ***14a***, *5-oxiranyl* ***8***, ***9*** *et* (E)*-5-(2-iodovinyl)-2,4-dichloro (ou diméthoxy)pyrimidines* ***11***, ***12*** *ont été synthétisées pour leur évaluation comme agents cytotoxiques. Les substituants sur les carbones 2 et 4 du noyau sont déterminants pour l'activité : les dérivés 2,4-dichlorés* ***6***, ***8*** *et* ***11*** *sont plus puissants (ED_{50} = 0,2–0,3 μg(ml) que les analogues 2,4-diméthoxypyrimidine correspondants* ***7***, ***9*** *et* ***12*** *(ED_{50} = 4–28 μg/ml), en référence au melphalan (ED_{50} = 0,15 μg/ml) dans le test* in vitro *L1210. Dans la série 2,4-dichlorée des composés* ***6***, ***8*** *et* ***11***, *le substituant du carbone 5 n'intervient pas sur l'activité. En revanche, dans la série 2,4-diméthoxypyrimidine, les substituants du C_5 agissent de façon significative sur l'activité, l'ordre de puissance relative étant oxiranyl* ***9*** *> -CH(OH)CH_2I* ***7*** *>* (E)*-CH=CHI* ***12*** *> CH(OH)CH_2I* ***13***, *CH(OH)CHBr(I)* ***14a*** *et CH(Br)CHOH(I)* ***14b***. *Le composé le plus actif, la* (E)*-5-(2-iodovinyl)-2,4-dichloropyrimidine* ***11*** *révèle une faible activité dans le test* in vivo *P388 (% T/C = 116 pour une dose de 10 mg/kg ip) en comparaison avec le composé de référence 5-fluorouracile (% T/C = 135 pour une dose de 20 mg/kg).*

cytotoxic agents / pyrimidines / oxiranes / 1-hydroxy-2-haloethanes

5-Substituted-uracil analogues have been investigated extensively in the search for effective non-toxic antitumor agents [1] that may be incorporatcd into DNA [2–5]. These compounds may enter pyrimidine metabolic pathways following bioconversion *via* salvage reactions to 5-substituted-2'-deoxyuridines, and then to the corresponding mono-, di- and triphosphates prior to incorporation into DNA [6]. Thus, 5-bromouracil **1a** and 5-vinyluracil **1b** are incorporated into the DNA of *Escherichia coli* [3]. Alternatively, 5-substituted-2'-deoxyuridine monophosphates could act as inhibitors of thymidylate synthetase (TS) to exhibit cytotoxic activity [7]. The antitumor activity of 5-fluorouracil **1c**, which is not incorporated into DNA [3], is due to its conversion to the nucleotide 5–fluoro-2'-deoxyuridine-5'-monophosphate **2a** which inhibits TS [8]. In contrast, there is evidence to suggest that the cytostatic effect of the thymidine analogue 5-hydroxymethyl-2'-deoxyuridine **2b** against Ehrlich ascites carcinoma cells is due to a cross-linking of **2b**, that has been incorporated in DNA, to chromatin proteins [9].

Activated alkylating agents that react with cellular nucleophiles such as L-cysteine, glutathione or sulfhydryl-containing enzymes, exhibit cytotoxic activity

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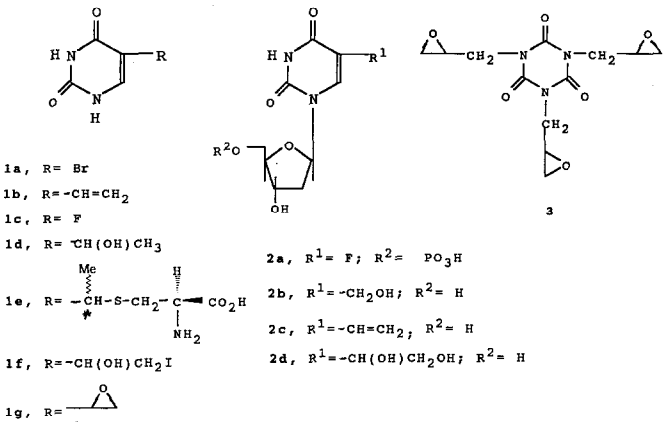
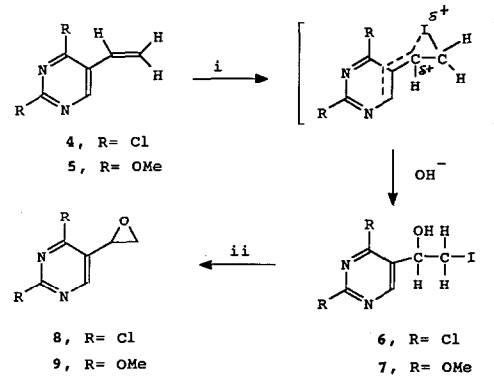
[10]. Many oxiranyl compounds, such as the 1,3,5-triglycidyl-*s*-triazinetrione **3**, exhibit significant anti-neoplastic activity [11]. It has also been demonstrated that 5-(1-hydroxyethyl)uracil **1d** reacts with L-cysteine to give a mixture of two separable diastereomers **1e** [12]. It is clear that 5-substituted-uracil derivatives offer the potential to exhibit cytotoxic activity *via* a number of mechanisms which are dependent upon the nature of the 5-substituent. We now describe the synthesis and cytotoxic activities of the structurally related 5-(1-hydroxy-2-haloethyl)- **6**, **7**, **13**, **14a**, 5-oxiranyl- **8**, **9** and (*E*)-5-(2-iodovinyl)-2,4-dichloro(or dimethoxy)pyrimidines **11**, **12** to acquire new structure–activity correlations for novel 5-substituted-uracil derivatives.



Chemistry

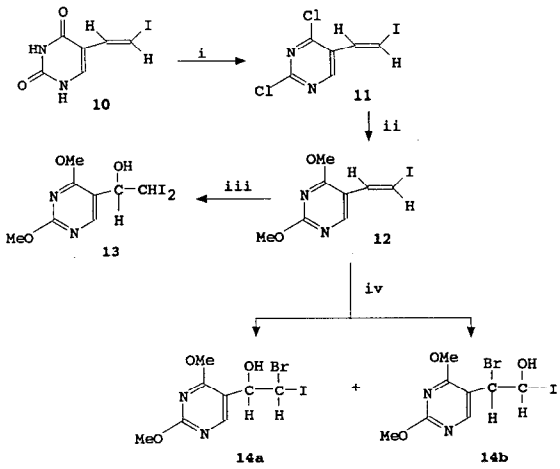
The reaction of 5-vinyluracil **1b** with phosphorous oxychloride in the presence of *N,N*-diethylaniline yielded 5-vinyl-2,4-dichloropyrimidine **4** in 63% yield. The subsequent reaction of **4** with sodium methoxide in methanol afforded 5-vinyl-2,4-dimethoxypyrimidine **5** in 71% yield. The 5-(1-hydroxy-2-iodoethyl)-2,4-dichloro(or dimethoxy)pyrimidines **6** and **7** were synthesized by reaction of **4** or **5** with iodine in the presence of the oxidizing agent iodic acid in 68 and 53% yields, respectively as illustrated in scheme 1. The ^{13}C NMR spectra of **6** and **7** provided conclusive evidence for the regiospecific addition of HOI across the vinylic double bond of **4** and **5**. The iodine atom is attached to a methylene carbon that exhibited a resonance at δ 11.47 and 13.19 whereas the hydroxyl substituent is attached to a methine carbon that exhibited a resonance at δ 68.04 and 67.77 for the respective compounds **6** and **7**. Treatment of **6** or **7** with NaOH or $Ca(OH)_2$ afforded the corresponding oxiranes **8** and **9** in 58 and 94% yields, respectively. In contrast, it was demonstrated in an earlier study that the reaction of 5-(1-hydroxy-2-iodoethyl)uracil **1f** with NaOMe [7], or with NaOH, $Ca(OH)_2$ or Na_2CO_3 under similar reaction conditions, did not afford 5-oxiranyluracil **1g**. Further attempts to synthesize 5-oxiranyluracil by oxidation of 5-vinyluracil **1b** using *m*-chloroperbenzoic acid or oxone were also unsuccessful in which case unreacted 5-vinyluracil was recovered. In a related reaction Jones *et al* observed that the oxidation of 5-vinyl-2'-deoxyuridine **2c** with *m*-chloroperbenzoic acid in water-tetrahydrofuran yielded 5-(1,2-dihydroxyethyl)-2'-deoxyuridine **2d** which was characterized as its tetra-*O*-acetate derivative. The formation of **2d** was postulated to arise from reaction of the intermediate oxirane that was readily opened by nucleophiles [13].

Reaction of (*E*)-5-(2-iodovinyl)uracil **10** with phosphorous oxychloride yielded (*E*)-5-(2-iodovinyl)-2,4-dichloropyrimidine **11** in 46% yield (see scheme 2). Treatment of **11** with sodium methoxide in methanol afforded (*E*)-5-(2-iodovinyl)-2,4-dimethoxypyrimidine **12** in 82% yield. The reaction of **12** with iodine and potassium iodate gave 5-(1-hydroxy-2,2-diiodoethyl)-2,4-dimethoxypyrimidine **13** (66%). In contrast, the related reaction of **12** with *N*-bromosuccinimide gave a mixture of the regioisomers **14a** and **14b** in a ratio of 1:1 (76%) that could not be separated by silica gel column or TLC chromatography. The ^1H NMR spectrum ($CDCl_3$) for the 1:1 mixture of **14a** and **14b** exhibited two multiplets attributed to the *CH*OH resonances at δ 5.1 and 5.14, two separate sets of closely spaced doublets assigned to the C*H*Br resonances at δ 5.88 and 6.0, and two closely spaced singlets for H-6 at δ 8.38 and 8.40. The ^{13}C NMR spectrum provided further evidence for the assigned structures based on the resonances at δ 19.13 (*C*HBrI of **14a**) and 50.46 (*C*HBr of **14b**), 74.36 and 73.31 (*C*HOH of **14a** and **14b**).



Reagents: i, I_2, KIO_3, H_2O/MeCN, 5N H_2SO_4
ii, NaOH, dioxane/H_2O (3:7, v/v) or $Ca(OH)_2$, H_2O

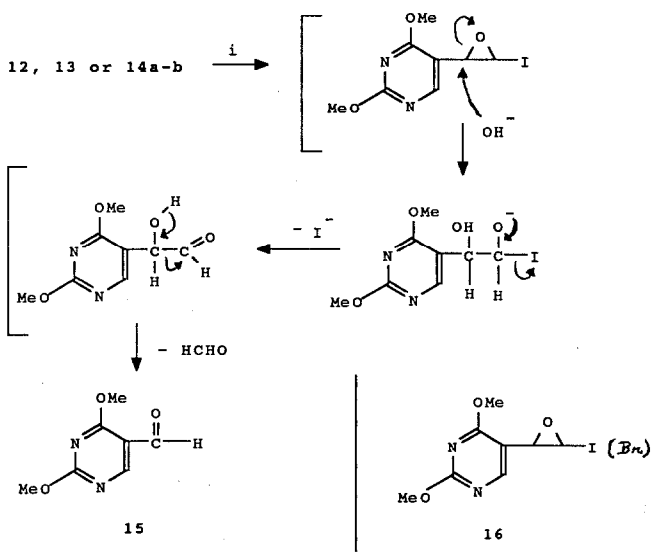
Scheme 1.



Reagents: i, $POCl_3$, *N*,*N*-diethylaniline
ii, NaOMe, MeOH
iii, I_2, KIO_3, 5N H_2SO_4, MeCN, 60°C
iv, *N*-bromosuccinimide, THF, HOAc, 25°C

Scheme 2.

Treatment of **13**, or a 1:1 mixture of **14a** and **14b**, with aqueous sodium hydroxide in dioxane yielded 5-formyl-2,4-dimethoxypyrimidine **15** in 77–80% yield, rather than the desired 5-(2-iodooxiranyl)-2,4-dimethoxypyrimidine **16** (see scheme 3). Oxidation of



Reagents: i, NaOH, dioxane/H_2O (**13** or **14a–b**);
oxone, disodium. EDTA, $NaHCO_3$, H_2O (**12**)

Scheme 3.

(*E*)-5-(2-iodovinyl)-2,4-dimethoxypyrimidine **12** with the strong oxidizing agent oxone monopersulfate ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) also afforded 5-formyl-2,4-dimethoxypyrimidine **15** (36%). A plausible mechanism for the formation of **15** in these reactions involves the formation of the unstable 5-(2-iodooxiranyl) intermediate **16**, or bromooxiranyl intermediate for the reaction employing **14a–b**, in which the oxirane ring is readily opened under the reaction conditions employed. The successive elimination of I^- and then HCHO would yield **15**. This mechanism is consistent with the observation that oxiranes bearing a good leaving substituent are very unstable and highly susceptible to hydrolysis [14].

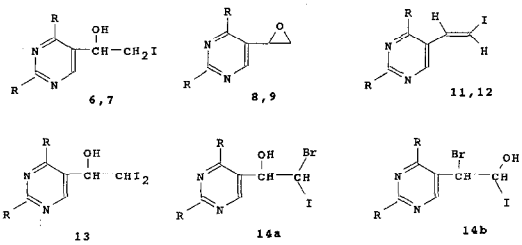
Structure–activity correlations

The 5-(1-hydroxy-2-iodoethyl) **6–7**, 5-oxiranyl **8–9**, (*E*)-5-(2-iodovinyl) **11–12**, 5-(1-hydroxy-2,2-diiodoethyl) **13**, 5-(1-hydroxy(bromo)-2-bromo(hydroxy)-2-iodoethyl **14a–b** derivatives of 2,4-dichloro (dimethoxy)pyrimidines were investigated in order to determine the effect of their nuclear and C-5 substituents upon cytotoxic activity. The cytotoxic activities acquired using the *in vitro* L1210 and KB, and the *in vivo* P388 leukemic, screens are summarized in table I.

The nuclear C-2 and C-4 substituents were significant determinants of activity since the 2,4-dichloro compounds **6**, **8** and **11** were more cytotoxic than the corresponding 2,4-dimethoxy analogues **7**, **9** and **12**. The 2,4-dichloro compounds exhibited cytotoxic activities (ED_{50} = 0.2 – 0.3 μg/ml) approaching that of the reference drug melphalan (ED_{50} = 0.15 μg/ml) in the *in vitro* L1210 screen. Their cytotoxicities were qualitatively similar in the *in vitro* KB screen (ED_{50} = 5–10 μg/ml) relative to the reference drug 5-fluorouridine (ED_{50} = 0.5 μg/ml). Within the 2,4-dichloro series of compounds **6**, **8** and **11**, the C-5 substituent was not a determinant of activity. The cytotoxic activities exhibited by **6** (R = $CH(OH)CH_2I$), **8** (R = oxiranyl) and **11** [R = (*E*)-CH=CHI] were similar. In contrast, in the less active 2,4-dimethoxy series of compounds, the differences in cytotoxic activities were large where the relative activity profile was **9** (R = oxiranyl) > **7** [R = $CH(OH)CH_2I$] > **12** [R = -CH=CHI] > **13** [R = $CH(OH)CHI_2$] and **14a–b** [R = CH(OH)CHBr(I), R = CH(Br)CHOH(I)]. A 2,4-dichloro **6** (ED_{50} = < 0.3 μg/ml) or 2,4-dimethoxypyrimidinyl ring **7** (ED_{50} = 15 μg/ml) also conferred superior cytotoxic activity in the L1210 screen relative to a 5-uracil ring **1f** (92% cell survival at 50 μg/ml) [7].

Compounds **6**, **8**, **9** and **11** were evaluated in the P388 *in vivo* screen since drugs that exhibit an ED_{50} < 5 μg/ml in the *in vitro* L1210 screen are considered to

Table I. Physical and cytotoxicity data for 5-(1-hydroxy-2-iodoethyl) (**6**, **7**), 5-oxiranyl (**8**, **9**), (*E*)-5-(2-iodovinyl) (**11**,**12**), 5-(1-hydroxy-2,2-diiodoethyl) (**13**), 5-(1-hydroxy-2-bromo-2-iodo) (**14a**) and 5-(1-bromo-2-hydroxy-2-iodoethyl) (**14b**) derivatives of 2,4-dichloro (or dimethoxy)pyrimidine.



No	R	*In vitro L1210 cytotoxicity ED_{50}, μg/ml*	*In vitro KB cytotoxicity ED_{50}, μg/ml*	*In vivo P388 activity % T/C, 20 mg/kg*[a]
6	Cl	< 0.3	5	105
7	OMe	15	> 10	ND[b]
8	Cl	0.3	5	102
9	OMe	4	> 100	114
11	Cl	0.2	10	116[c]
12	OMe	28	> 10	ND
13	OMe	> 50	> 100	ND
14a–b[d]	OMe	> 50	> 100	ND
FURD[e]			0.5	ND
FU[f]				135
Melphalang		0.15		

[a]% T/C = treated/control; [b]ND = not determined; [c]Tested at a dose of 10 mg/kg ip; [d]Tested as a 1:1 mixture of **14a** and **14b**; [e]FUDR = 5-fluorouridine; [f]FU = 5-fluorouracil; [g]4-[*N*-bis-2-(chloroethyl)amino]phenylalanine.

be active cytotoxic agents. In contrast to the *in vitro* test results, all compounds tested (**6**, **8**, **9**, **11**) were inactive in the P388 *in vivo* screen. The most potent compound (*E*)-5-(2-iodovinyl)-2,4-dichloropyrimidine **11** (% T/C = 116 at a 10 mg/kg ip dose) exhibited weak activity relative to the reference drug 5-fluorouracil (% T/C = 135 at a 20 mg/kg dose).

Experimental protocols

Chemistry

Melting points were determined with a Buchi capillary apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were measured on a Bruker AM-300 spectrometer. ^{13}C NMR (*J* modulated spin echo) spectra were determined in all instances where methyl and methine carbon resonances appear as positive peaks, and methylene and quaternary carbon resonances appear as negative peaks. Chemical shifts are given in ppm relative to TMS as internal standard. Mass spectra were recorded on a Hewlett-Packard 5995A (EI) spectrometer. Thin layer chromatography was performed using Whatman MK6F silica gel microslides (250 μm thickness). Silica gel column chromatography was carried out using Merck 7734 silica gel (100–200 μm particle size). 5-Vinyluracil **1b** [7], 5-(1-hydroxy-2-iodoethyl)uracil **1f** [7] and (*E*)-5-(2-iodovinyl)uracil **10** [15] were prepared using literature procedures.

*5-Vinyl-2,4-dichloropyrimidine **4***

A mixture of 5-vinyluracil (0.24 g, 1.74 mmol), $POCl_3$ (4 ml) and *N,N*-diethylaniline (0.4 ml) was refluxed for 30 min. The volatile components were removed *in vacuo*, the oil-like residue remaining was dissolved in chloroform and washed with cold water. The chloroform extract was dried (Na_2SO_4) and the residue obtained, after removal of the solvent, was purified by silica gel column chromatography using *n*-hexane:ethyl acetate (98:2, v/v) as eluant. Removal of the solvent from the fractions containing the product yielded **4** as a yellowish oil (0.192 g, 63.2%) which was used immediately in subsequent reactions; ^{1}H NMR (Me_2SO-d_6) δ: 9.14 (s, 1H, H-6), 6.85 (dd, J_{trans} = 18 Hz, J_{cis} = 12 Hz, 1H, C*H* = CHH'); 6.24 (d, J_{trans} = 18 Hz, 1H, CH=C*H*H'); 5.74 (d, *Jcis* = 12 Hz, CH=C*H*H').

*5-Vinyl-2,4-dimethoxypyrimidine **5***

Sodium methoxide (0.97 g, 18 mmol) was added to a solution of **4** (0.095 g, 5.45 mmol) in methanol (15 ml) at 25°C with stirring. The reaction mixture was stirred at 25°C for 52 h. After neutralization to pH 7 using acidic resin (Dowex 50X 8-200) and removal of the solvent *in vacuo*, the residue obtained was purified by silica gel column chromatography using *n*-hexane:ethyl acetate (97:3, v/v) as eluant to afford **5** as a viscous oil [16] (0.065 g, 71.5% yield); ^{1}H NMR (Me_2SO-d_6) δ: 8.52 (s, 1H, H-6); 6.67 (dd, J_{trans} = 18 Hz, J_{cis} = 12 Hz, 1H, C*H* = CHH'); 5.92 (d, J_{trans} = 18 Hz, 1H, CH=C*H*H'); 5.34 (d, J_{cis} = 12 Hz, 1H, CH=C*H*H'); 4.02 and 3.94 (two s, 3H each, OMe). Mass calcd for $C_8H_{10}N_2O_2$: 166. Found: 166.

*5-(1-Hydroxy-2-iodoethyl)-2,4-dichloropyrimidine **6***

A mixture of 5-vinyl-2,4-dichloropyrimidine **4** (0.04 g, 0.228 mmol), iodine (0.025 g, 0.2 mmol), potassium iodate (0.015 g, 0.07 mmol), water (2 ml), acetonitrile (1 ml) and sulfuric acid (20 μl of 5 N) was stirred at 60°C for 6 h. The reaction mixture was concentrated *in vacuo* and the residue was partitioned between chloroform and water. The chloroform soluble material was then separated by silica gel column chromatography using *n*-hexane:ethyl acetate (95:5, v/v) as eluant to yield **6** (0.05 g, 68%), mp = 100–102°C; ^{1}H NMR ($CDCl_3$) δ: 8.80 (s, 1H, H-6); 5.04 (m, 1H, C*H*OH); 3.66 (d, J_{gem} = 10.9 Hz of d, J_{vic} = 3.0 Hz, 1H, CH*H*'I); 3.34 (d, J_{gem} = 10.9 Hz of d, J_{vic} = 7.1 Hz, 1H, C*H*H'I); 2.68 (d, J_{CH_2OH} = 4.1 Hz, 1H, CHO*H*, exchanges with deuterium oxide); ^{13}C NMR ($CDCl_3$) δ: 159.87 (C-4); 159.55 (C-2); 159.0 (C-6); 131.81 (C-5); 68.04 (*C*HOH); 11.47 (CH_2I). Mass calcd for $C_6H_5{}^{35}Cl_2IN_2O$: 318. Found: 318. Mass calcd for $C_6H_5{}^{37}Cl_2IN_2O$: 322. Found: 322. Anal calcd for $C_6H_5Cl_2IN_2O$: C: 22.59; H: 1.58: N: 8.78. Found: C: 22.76; H: 1.54; N: 8.91.

*5-(1-Hydroxy-2-iodoethyl)-2,4-dimethoxypyrimidine **7***

A mixture of **5** (0.05 g, 0.3 mmol), iodine (0.038 g, 0.3 mmol), potassium iodate (0.015 g, 0.07 mmol), water (2 ml), acetonitrile (0.5 ml) and sulfuric acid (25 μl of 5 N) was heated at 55°C with stirring for 1 h. Removal of the solvent *in vacuo*, extraction of the residue with chloroform, drying the chloroform extract (Na_2SO_4) and evaporation of the solvent gave a residue. Purification of this residue by silica gel column chromatography using *n*-hexane:ethyl acetate (85:15, v/v) as eluant afforded **7** as crystals (0.049 g, 53.3%), mp = 68–70°C; ^{1}H

NMR ($CDCl_3$) δ: 8.37 (s, 1H, H-6); 4.89 (m, 1H, C*H*OH); 4.07 and 4.04 (two s, 3H each, OMe); 3.63 (d, J_{gem} = 10.7 Hz of d, J_{vic} = 3.12 Hz, 1H, CH*H'*I); 3.44 (d, J_{gem} = 10.7 Hz of d, J_{vic} = 7.31 Hz, 1H, C*H*H'I); 2.64 (d, $J_{CH,OH}$ = 5.5 Hz, 1H, CHO*H*, exchanges with deuterium oxide); ^{13}C NMR ($CDCl_3$) δ: 167.89 (C-4); 164.97 (C-2); 156.43 (C-6); 114.64 (C-5); 67.77 (*C*HOH); 54.83 (O*C*H_3); 54.07 (O*C*H_3); 13.19 (*C*H_2I). Mass calcd for $C_8H_{11}IN_2O_3$: 310. Found: 310. Anal calcd for $C_8H_{11}IN_2O_2$: C: 30.98; H: 3.57; N: 9.03. Found: C: 30.81; H: 3.66; N: 8.99.

5-Oxiranyl-2,4-dichloropyrimidine ***8***

A solution of 5-(1-hydroxy-2-iodoethyl)-2,4-dichloropyrimidine **6** (0.058 g, 0.182 mmol) in dioxane-water (3:7, v/v, 3 ml) and sodium hydroxide (0.01 g, 0.25 mmol) was stirred at 25°C for 30 min. The solvent was removed *in vacuo* and the residue was extracted with chloroform (25 ml). Removal of the solvent *in vacuo* afforded a residue which was purified by silica gel column chromatography. Elution with *n*-hexane:ethyl acetate (96:4, v/v) gave **8** (0.02 g, 57.6%), mp = 52–53°C; ^1H NMR ($CDCl_3$) δ: 8.46 (s, 1H, H-6); 4.13 (dd, J_{vic} = 3.6 Hz, J_{vic} = 2.4 Hz, 1H, oxiranyl CH); 3.30 (dd, J_{gem} = 5.4 Hz, J_{vic} = 3.6 Hz, 1H CH*H'*); 2.77 (dd, J_{gem} = 5.4 Hz, J_{vic} = 2.4 Hz, 1H, C*H*H'). Mass calcd for $C_6H_4{}^{35}Cl_2N_2O$: 190. Found: 190. Mass calcd for $C_6H_4{}^{37}Cl_2N_2O$: 194. Found: 194. Anal calcd for $C_6H_4Cl_2N_2O$: C: 37.72; H: 2.10; N: 14.66. Found: C: 37.66; H: 2.25; N: 14.24.

5-Oxiranyl-2,4-dimethoxypyrimidine ***9***

Calcium hydroxide (0.44 g, 6 mmol) was added to **7** (0.112 g, 0.36 mmol) in chloroform (20 ml) and water (10 ml). The reaction was allowed to proceed with stirring at 25°C for 3 h. The chloroform layer was separated, dried (Na_2SO_4) and the solvent was removed *in vacuo*. Recrystallization of the residue obtained from chloroform-hexane yielded **9** (0.062 g, 94%), mp = 52°C; ^1H NMR ($CDCl_3$) δ: 8.06 (s, 1H, H-6); 4.02 and 3.98 (two s, 3H each, OMe); 3.93 (dd, J_{vic} = 3.8 Hz, J_{vic} = 2.4 Hz, 1H, oxiranyl CH), 3.09 (dd, J_{gem} = 5.7 Hz, J_{vic} = 3.8 Hz, 1H, CH*H'*); 2.78 (dd, J_{gem} = 5.7 Hz, J_{vic} = 2.4 Hz, 1H, C*H*H'). Mass calcd for $C_8H_{10}N_2O_3$: 182. Found: 182. Anal calcd for $C_8H_{10}N_2O_3$: C: 52.73; H: 5.53; N: 15.38. Found: C: 53.24; H: 5.46; N: 14.85.

(E)-5-(2-Iodovinyl)-2,4-dichloropyrimidine ***11***

A mixture of **10** (0.475 g, 1.8 mmol), $POCl_3$ (4 ml) and *N,N*-diethylaniline (0.4 ml) was refluxed for 30 min. Isolation and purification of the product, as described for **4**, afforded **11** as light yellow crystals (0.25 g, 46%), mp = 72–73°C; ^1H NMR ($CDCl_3$) δ: 8.62 (s, 1H, H-6); 7.62 (d, *J* = 15 Hz, 1H, CH=C*H*I); 7.29 (d, *J* = 15 Hz, 1H, C*H*=CHI). Mass calcd for $C_6H_3{}^{35}Cl_2IN_2$: 300. Found: 300. Mass calcd for $C_6H_3{}^{37}Cl_2IN_2$: 304. Found: 304. Anal calcd for $C_6H_3Cl_2IN_2$: C: 23.94; H: 1.00; N: 9.31. Found: C: 24.12; H: 0.97; N: 9.09.

(E)-5-(2-Iodovinyl)-2,4-dimethoxypyrimidine ***12***

Reaction of **11** (0.05 g, 0.165 mmol) in methanol (20 ml) with sodium methoxide (0.24 g, 4.5 mmol), and isolation of the product as described for the preparation of **3**, afforded **12** as colorless crystals (0.04 g, 82%), mp = 89–90°C; ^1H NMR ($CDCl_3$) δ: 8.18 (s, 1H, H-6); 7.33 (d, *J* = 15 Hz, 1H, CH=C*H*I); 7.04 (d, *J* = 15 Hz, 1H, C*H*=CHI); 4.07 and 4.04 (two s, 3H each, OMe). Mass calcd for $C_8H_9IN_2O_2$: 292. Found: 292. Anal calcd for $C_8H_9IN_2O_2{\cdot}1/4H_2O$: C: 32.39; H: 3.22; N: 9.44. Found: C: 32.35; H: 3.00; N: 9.09.

5-(1-Hydroxy-2,2-diiodoethyl)-2,4-dimethoxypyrimidine ***13***

A solution of **12** (0.05 g, 0.172 mmol), iodine (0.022 g, 0.17 mmol), potassium iodate (0.01 g, 0.047 mmol), acetonitrile (3 ml), water (2 ml) and sulfuric acid (15 µl of 5 N) was maintained at 60°C for 4 h with stirring. Removal of the solvent *in vacuo* gave a residue which was purified by silica gel column chromatography using hexane:ethyl acetate (85:15, v/v) as eluant to yield **13** (0.05 g, 66.8%) as a white solid after recrystallization from hexane, mp = 118–119°C; ^1H NMR ($CDCl_3$) δ: 8.46 (s, 1H, H-6); 5.58 (d, *J* = 3.6 Hz, 1H, C*H*I_2); 4.72 (d, $J_{CH,OH}$ = 5.0 Hz of d, $J_{CH,CH}$ = 3.6 Hz, 1H, C*H*OH); 4.06 and 4.04 (two s, 3H each, OMe); 2.92 (d, *J* = 5.0 Hz, 1H, CHO*H*, exchanges with deuterium oxide). Mass calcd for $C_8H_{10}I_2N_2O_3$: 436. Found: 436. Anal calcd for $C_8H_{10}I_2N_2O_3$: C: 22.03; H: 2.31; N: 6.42. Found: C: 22.42; H: 2.23; N: 7.08.

5-(1-Hydroxy-2-bromo-2-iodoethyl)-2,4-dimethoxypyrimidine ***14a*** *and 5-(1-bromo-2-hydroxy-2-iodoethyl)-2,4-dimethoxypyrimidine* ***14b***

A mixture of *N*-bromosuccinimide (0.03 g, 0.17 mmol), **12** (0.05 g, 0.17 mmol), water (2 ml), THF (2 ml) and glacial acetic acid (10 µl) was allowed to stir at 25°C for 24 h. Removal of the solvent *in vacuo* and purification of the product by elution from a silica gel column using hexane:ethyl acetate (88:12, v/v) as eluant yielded a mixture of the two regioisomers **14a** and **14b** in a ratio of 1:1, as a white solid (0.05 g, 76%) after recrystallization form hexane, mp = 101–102°C; ^1H NMR ($CDCl_3$) δ: 8.38 and 8.40 (two s, 2H total, H-6); 6.0 (d, *J* = 3.68 Hz, 1H, C*H*Br in one regioisomer); 5.88 (d, *J* = 3.68 Hz, 1H, C*H*Br in one regioisomer); 5.14 (d, $J_{CH,CH}$ = 3.68 Hz of d, $J_{CH,OH}$ = 5 Hz, 1H, C*H*OH in one regioisomer); 5.1 (d, $J_{CH,CH}$ = 3.68 Hz of d, $J_{CH,OH}$ = 5.0 Hz, 1H, C*H*OH in one regioisomer); 4.02 and 4.0 (two s, 12H total, OMe); 2.95 and 2.91 (two d, $J_{CH,OH}$ = 5 Hz each, 2H total, OH, exchange with deuterium oxide); ^{13}C NMR ($CDCl_3$) δ: 19.13 (*C*HBrI of **14a**); 50.46 (*C*HBr of **14b**); 55.01 and 54.30 (O*C*H_3 of **14a** and **14b**); 74.36 and 73.31 (*C*HOH of **14a** and **14b**); 112.01 (C-5); 158.07 (C-6); 167.69 (C-4). Mass calcd for $C_8H_{10}{}^{79}BrIN_2O_3$: 388. Found: 388. Anal calcd for $C_8H_{10}BrIN_2O_3{\cdot}1H_2O$: C: 23.58; H: 2.94; N: 6.87. Found: C: 23.55; H: 2.73; N: 7.18.

5-Formyl-2,4-dimethoxypyrimidine ***15****. Method A, synthesis from* ***13***

Sodium hydroxide (3.2 mg, 0.08 mmol) was added to a solution of **13** (0.011 g, 0.025 mmol) in dioxane (2 ml) and water (80 µl) and the reaction was allowed to proceed at 25°C for 90 min with stirring. The solvent was removed *in vacuo* and the residue was dissolved in methanol. Silica gel (2 g) was added to this methanol solution and the solvent was removed. This material was applied to the top of a silica gel column. Elution with ethyl acetate:hexane (5:95, v/v) and collection of the fractions containing the product afforded **15** as a white solid (3.4 mg, 80%), mp = 120–121°C (Lit [17] 123°C); ^1H NMR ($CDCl_3$) δ: 10.20 (s, 1H, CHO); 8.80 (s, 1H, H-6); 4.10 and 4.15 (two s, 3H each, OMe); ^{13}C NMR ($CDCl_3$) δ: 55.61 and 54.48 (O*C*H_3); 112.29 (C-5); 161.37 (C-6); 167.55 (C-2); 171.11 (C-4); 186.31 (*C*HO). Mass calcd for $C_7H_8N_2O_3$: 168. Found: 168. Anal calcd for $C_7H_8N_2O_3$: C: 49.99; H: 4.79; N: 16.66. Found: C: 50.23; H: 4.65; N: 16.45.

Method B, synthesis from ***14a–b***

Sodium hydroxide (5 mg, 0.125 mmol) was added to a solution of **14a–b** (15 mg, 0.038 mmol) in dioxane (2 ml) and water (1 ml), and the reaction was allowed to proceed at 25°C for 90 min with stirring. Removal of the solvent *in vacuo* and purification of the product by elution from a silica gel column

using hexane:ethyl acetate (95:5, v/v) as eluant yielded **15** as a white solid (5 mg, 77%), which was identical (mp, ^{1}H NMR, MS) to **15** described under Method A.

Method C, synthesis from ***12***
Sodium bicarbonate (18 mg, 0.21 mmol) and the water (0.3 ml) was added slowly to a stirred slurry of **12** (15 mg, 0.05 mmol) in acetone (0.1 ml). A solution of oxone monopersulfate ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) (0.03 g) in 4 x 10^{-4} M aqueous disodium EDTA (0.12 ml) was added dropwise during 30 min to the thick mixture with stirring. After the addition was complete, the mixture was stirred at 25°C for 2 h. Removal of the solvent *in vacuo*, extraction of the residue with chloroform, drying the chloroform extract (Na_2SO_4) and removal of the solvent gave a residue. Purification of this residue by silica gel column chromatography using *n*-hexane:ethyl acetate (96:4, v/v) as eluant afforded **15** as a white solid (3 mg, 36%), which was identical (mp, ^{1}H NMR, MS) to **15** described under Method A.

Pharmacology

In vitro L1210 cytotoxic screen
Mouse L1210 leukemia cells were cultivated as a suspension in Fischer's medium supplemented with 10% heat-inactivated horse serum and incubated at 37°C in a humidified 5% CO_2 atmosphere to prepare a cell stock solution. The number of cells/ml of medium was determined using a Model ZF Coulter Counter 48 h after incubation. The test compound was dissolved in ethanol:water (5:95, v/v) (**7**, **9**) or ethanol (**6**, **8**, **11–14**), and 20 µl of this solution was added to test wells containing 2 ml of suspended L1210 cells (10^5 cells/ml) such that 2 ml of the cell suspension had a test compound concentration of 50, 10 and 1 µg/ml of medium, respectively. Control wells were identical, except that the test compound was absent. Melphalan, the reference compound, had the following test compound concentrations (10, 1, 0.5, 0.1 and 0.05 µg/ml of medium). All test and controls were grown in triplicate. The % cell survival was calculated using the formula: % survival = $(T_{48}-T_0)/(C_{48}-C_0)$ x 100; where T_{48} is the number of cells/ml for each test drug concentration at 48 h, T_0 is the number for test wells at time zero (normally 10^5), C_{48} is the number for the control at 48 h, and C_0 is the number for the control at time zero (normally, $T_0 = C_0 = 10^5$ cells/ml). Compounds exhibiting an $ED_{50} < 5$ µg/ml are considered to be active in this screen.

In vitro KB cytotoxic screen
A solution (50 µl) of the test compound in aqueous ethanol (1:1, v/v) which provided final test compound concentrations of 100, 10, 5, 1, 0.5, 0.1 and 0.05 µg/ml, respectively was added to wells of a 96 well-plate. Each well was seeded with a suspension (100 µl) of KB cells (ATCC-CCL17) and the plates were incubated for 3 days at 37°C in a CO_2 incubator. Cytotoxicity toward KB cells was determined using the neutral red dye uptake assay [18]. Thus, 25 µl of a neutral red solution (0.05%) was added to each well, the plates were incubated at 37°C for 2.5 h and then washed with phosphate buffer solution. Washing buffer (50 µl) was added to each well which was incubated for 2 h and the plates were read at 540 nm using a spectrophotometer. The concentration of test compound required to reduce cell survival by 50% of the control value was determined to be the ED_{50}.

In vivo P388 lymphocytic screen
Compounds were tested for their ability to prolong the survival of $B_6D_2F_1$ mice (6 per group) receiving an implant of P388 mouse leukemia cells (10^6 cells in 0.2 ml saline) ip on day 0. The test compound was constituted in saline:ethanol (6:1, v/v) and injected (20 mg/kg ip) on days 1 through 5 (0.2 ml). The mice were observed until death, and the mean survival times were compared to controls receiving the same number of cells on day 0 and sterile saline (0.2 ml) for the five following days. Significant *in vivo* antineoplastic activity is ascribed to compounds exhibiting a % T/C > 125.

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