Synthesis and Antiviral Activity of 6-Benzyl Analogs of 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) as Potent and Selective Anti-HIV-1 Agents

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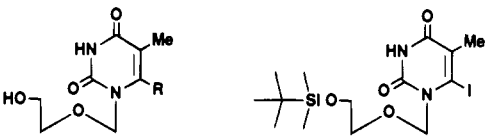
Several 6-benzyl analogs of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (**1**; HEPT) were synthesized and evaluated for their anti-HIV-1 activity. LDA (lithium diisopropylamide) lithiation of 5-ethyluracil derivatives **7** and **8** and subsequent reaction with an aryl aldehyde gave 6-(arylhydroxymethyl)-5-ethyluracil derivatives **9**–**12**. 6-(Arylhydroxymethyl)-5-isopropyluracil derivatives **15**–**18** were prepared from the 5-isopropyl-2-thiouracil derivatives **13** and **14** by the above procedure following oxidative hydrolysis of the thione. Preparation of the target 5-alkyl-1-(alkoxymethyl)-6-benzyluracil derivatives **27**–**34** was carried out by acetylation of **9**–**14** followed by Pd-catalyzed hydrogenolysis. The 1-butyl- (**37** and **39**) and 1-(2-methoxyethyl)- (**38** and **40**) 5-alkyl-6-benzyluracils were synthesized by 1-alkylation of the 3-phenacyl derivatives **35** and **36** with alkyl halides followed by deprotection of the 3-phenacyl group. Compounds synthesized in this study inhibited HIV-1 replication in MT-4 cells in the submicromolar to namomolar concentration range. From this series of compounds, 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (**33**) was selected for clinical evaluation.

The limitation of nucleoside analogs has encouraged a search for non-nucleoside reverse transcriptase (RT) inhibitors (NNRTI).[1-7] We have reported that 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (1; HEPT) has potent and selective *in vitro* activity against HIV-1.[8] In contrast to known nucleoside analogs, HEPT itself proved inhibitory only to HIV-1 RT.[9]

It has previously been shown that the analog in which the 6-phenylthio group of HEPT has been replaced with a benzyl group also has activity against HIV-1 in MT-4 cells.[10] In the present article, we report further modifications of this analog, 6-benzyl-1-[(2-hydroxyethoxy)-methyl]thymine (**2**), which provide highly potent anti-HIV-1 agents.

Chemistry

We have described the structure–activity relationships of 6-substituted derivatives of HEPT, wherein a ring structure at the C-6 position of the pyrimidine moiety was an important determinant for the anti-HIV-1 activity.[10] To investigate the optimum distance between these two ring systems for activity, preparation of the 6-phenyl and 6-phenylethyl analogs was carried out. Treatment of 1-[[2-[(*tert*-butyldimethylsilyl)oxy]-ethoxy]methyl]-6-iodothymine (**3**)[10] with Ph_4Sn (5 equiv) in dioxane in the presence of $(Ph_3P)_2PdCl_2$ (0.1 equiv),[11] followed by acidic deprotection of the *tert*-butyldimethylsilyl group, gave the desired 6-phenyl derivative **4** in 30% yield. The 6-phenylethyl derivative **6** was synthesized by palladium-catalyzed hydrogenation of the phenylethynyl derivative **5**.[10]



1 R = SPh; HEPT
2 R = CH_2Ph
4 R = Ph
5 R = C≡CPh
6 R = CH_2CH_2Ph

3

It has been shown that direct benzylation of the C-6-lithiated species of pyrimidine acyclonucleosides with benzyl bromide resulted in complete recovery of starting material,[10,12] due to α-elimination of the benzyl bromide which forms a carbene.[12] Therefore, the preparation of 6-benzyl derivatives has been achieved by the following sequence of reactions: (1) reaction of the C-6-lithiated species with benzaldehyde, (2) acetylation of the resulting 6-phenylhydroxymethyl derivatives, and (3) Pd–C-catalyzed hydrogenolysis of the CH(Ph)–OAc bond.[10,12]

In the present study, syntheses of 6-benzyl derivatives **27**–**34** were carried out according to the above-mentioned approach (Scheme 1). Thus, the 5-ethyluracil derivatives **7** and **8**[13] were treated with LDA (lithium diisopropylamide; 2.2 equiv) in THF below −70 °C for 1 h, and the resulting C-6-lithiated species were then allowed to react with the aryl aldehyde (1.5 equiv). This reaction gave 6-(arylhydroxymethyl)-5-ethyluracil derivatives **9**–**12**, which were not isolated but acetylated with Ac_2O in pyridine. The acetates **19**–**22** were obtained in 75–84% yield by this procedure.

In contrast, the reaction of the C-6-lithiated 5-isopropyluracil derivatives with benzaldehyde gave 6-phenyl-

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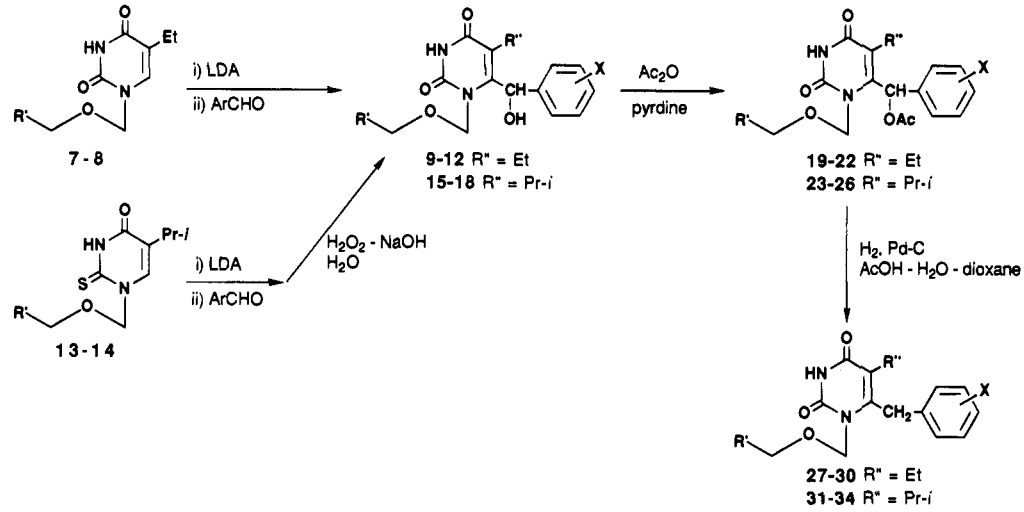
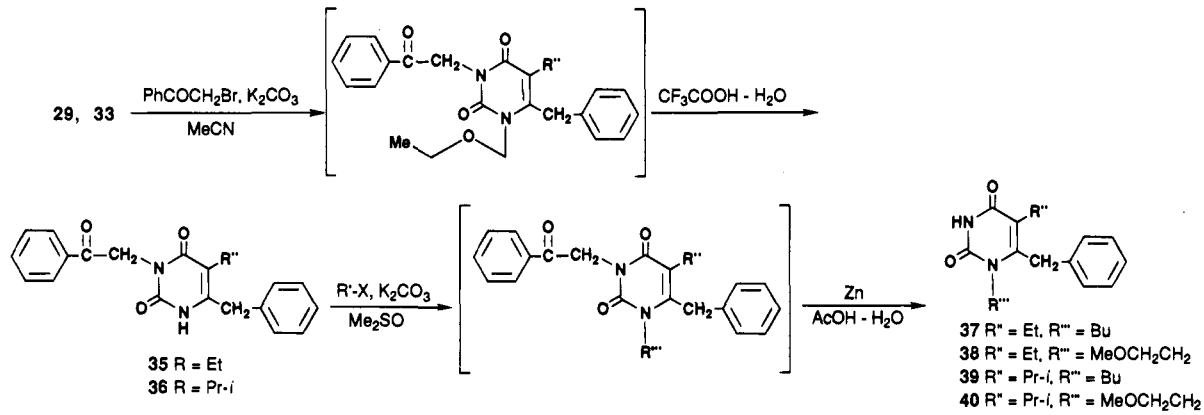
Scheme 1[a]



[a] For **7**, **9**, **10**, **13**, **15**, **16**, **19**, **20**, **23**, and **24**, R′ = $TBDMSOCH_2$. For **8**, **11**, **12**, **14**, **17**, **18**, **21**, **22**, **25**, **26**, **29**, **30**, **33**, and **34**, R′ = Me. For **27**, **28**, **31**, and **32**, R = $HOCH_2$. X = H or 3,5-di-Me (see the Experimental Section).

Scheme 2



hydroxymethyl derivatives in only low yields.[14] Thus the 5-isopropyl-2-thiouracil derivatives **13** and **14**[13] were examined. The LDA lithiation of **13** and **14** followed by treatment with an aryl aldehyde gave 6-(arylhydroxymethyl)-5-isopropyl-2-thiouracils. Oxidative hydrolysis (H_2O_2 in aqueous NaOH) of the thione function afforded the 5-isopropyl-6-(arylhydroxymethyl)uracil derivatives **15**–**18**.[15] Acetylation of **15**–**18** gave acetates **23**–**26** in 40–50% yield from **13** and **14**.

When **19**–**26** were subjected to hydrogenolysis in the presence of 10% Pd–C (in AcOH–H_2O–dioxane, at 60 °C, 1 atm, for 15 h), the expected 6-benzyl derivatives **27**–**34** were obtained.

We have reported that the 1-ethyl and 1-butyl derivatives of HEPT exhibit anti-HIV-1 activity, suggesting that the oxygen atom at the 2-position in the acyclic portion of HEPT is not essential.[13] To investigate this structure–activity relationship in the class of 6-benzyl derivatives, N-1 modification of **29** and **33** was carried out. The N-3 position of **29** and **33** was protected with the phenacyl group. After acidic treatment of the reaction mixture with CF_3COOH–H_2O, **35** and **36** were obtained. Alkylation of **35** and **36** using BuI or $MeOCH_2$-CH_2Br in the presence of K_2CO_3, followed by deprotection of the N-3 phenacyl group, gave the target 1-butyl (**37** and **39**) and 1-(2-methoxyethyl) (**38** and **40**) derivatives.

Results of the Anti-HIV Assay and Discussion

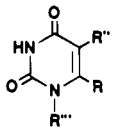
Anti-HIV-1 activity and cytotoxicity of HEPT analogs synthesized in the present study are summarized in Table 1 together with those of **2**, HEPT, and AZT. The procedure to measure anti-HIV-1 activity has been described previously.[16] The $HTLV\text{-}III_B$ strain of HIV-1 and MT-4 cells were used for the assay.

The 6-phenyl (**4**) and 6-phenylethyl (**6**) analogs of **2** were inactive against HIV-1 replication in MT-4 cells. These results suggested that the distance between the pyrimidine and the phenyl ring is an important criterion for activity.

Our previous studies concerning the structure–activity relationships of HEPT analogs suggested that the following modifications potentiated their anti-HIV-1 activity: (1) replacement of the 5-methyl group in the base moiety with a bulkier alkyl group,[17] (2) substitution at the 3- and 5- positions of the 6-phenyl ring with two methyl groups,[17] and (3) removal of the hydroxyl group in the (2-hydroxyethoxy)methyl side chain.[13] The molecular design of **28**–**34** and **37**–**40** was based on these findings.

Replacement of the 5-methyl group of **2** with a 5-ethyl (**27**, EC_{50}: 0.35 μM) or a 5-isopropyl (**31**, EC_{50}: 0.063 μM) group potentiates the original activity of **2** 66 and 365 times, respectively. The 1-ethoxymethyl derivatives **29** and **33** and the 6-(3,5-dimethylbenzyl) derivatives **28**, **30**, **32**, and **34** proved to be highly potent and selective inhibitors of HIV-1 in MT-4 cells. Their EC_{50}

Table 1. Inhibition of HIV-1 Replication in MT-4 Cells by 6-Benzylpyrimidine Derivatives



compd	R	R″	R‴	EC_{50},[a] μM	CC_{50},[b] μM	SI[c]
4	Ph	Me	$HOCH_2CH_2OCH_2$	>166	166	<1
6	CH_2CH_2Ph	Me	$HOCH_2CH_2OCH_2$	>444	444	<1
27	CH_2Ph	Et	$HOCH_2CH_2OCH_2$	0.35	391	1100
28	CH_2Ph(3,5-di-Me)	Et	$HOCH_2CH_2OCH_2$	0.013	281	22 000
29	CH_2Ph	Et	$EtOCH_2$	0.041	245	6000
30	CH_2Ph(3,5-di-Me)	Et	$EtOCH_2$	0.0016	207	130 000
31	CH_2Ph	*i*-Pr	$HOCH_2CH_2OCH_2$	0.063	295	4700
32	CH_2Ph(3,5-di-Me)	*i*-Pr	$HOCH_2CH_2OCH_2$	0.0027	221	82 000
33	CH_2Ph	*i*-Pr	$EtOCH_2$	0.0042	186	44 000
34	CH_2Ph(3,5-di-Me)	*i*-Pr	$EtOCH_2$	0.0006	43	72 000
37	CH_2Ph	Et	Bu	0.21	>500	>2400
38	CH_2Ph	*i*-Pr	Bu	0.042	58	1400
39	CH_2Ph	Et	$MeOCH_2CH_2$	0.25	362	1400
40	CH_2Ph	*i*-Pr	$MeOCH_2CH_2$	0.052	195	3800
2	CH_2Ph	Me	$HOCH_2CH_2OCH_2$	23	352	15
HEPT	SPh	Me	$HOCH_2CH_2OCH_2$	7.0	740	106
AZT				0.003	7.8	2600

[a] Effective concentration of compound required to achieve 50% protection of MT-4 cells against the cytopathic effect of HIV-1. [b] Cytotoxic concentration of compound required to reduce the viability of mock-infected MT-4 cells by 50%. [c] Selectivity index: ratio of CC_{50} to EC_{50}.

Table 2. Inhibition of HIV-1 and HIV-2 Replication in MT-4 Cells and PBLs by **33** and AZT[a]

compd	virus	strain	cell	EC_{50}, μM	CC_{50}, μM
33	HIV-1	$HTLV\text{-}III_B$	PBL	0.004	90
	HIV-1	A012D	MT-4	0.003	102
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	>102	102
AZT	HIV-1	$HTLV\text{-}III_B$	PBL	0.002	12
	HIV-1	A012D	MT-4	0.3	7.8
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	0.0028	7.8

[a] The antiviral activity and cytotoxicity of the compounds were expressed as the EC_{50} for virus-infected cells and the CC_{50} for mock-infected cells, respectively.

Table 3. Inhibitory Effect of **33** and AZT on Colony Formation of Murine Bone Marrow Progenitor Cells

		number of colonies[a]	
compd	conc (μM)	GM-CFS[b]	IL-3[b]
control		89 ± 5	137 ± 14
33	50	57 ± 4**	109 ± 2*
	5	77 ± 6	137 ± 11
	0.5	75 ± 3*	137 ± 10
	0.05	89 ± 3	136 ± 5
AZT	50	14 ± 3**	10 ± 2**
	5	47 ± 3**	74 ± 5**
	0.5	70 ± 6**	109 ± 8**
	0.05	85 ± 7	141 ± 4

[a] Values are means ± deviations in triplicate experiments. [b] See the Experimental Section. *: $p < 0.05$. **: $p < 0.01$.

values were within the nanomolar concentration range. Furthermore, **28**, **30**, and **32**–**34** had very large selectivity indices (SIs, ratios of CC_{50} to EC_{50}) which were 22 000, 130 000, 82 000, 44 000, and 72 000, respectively. The 1-butyl derivatives **37** and **39** and the 1-(2-methoxyethyl) derivatives **38** and **40** were also as potent as their 1-(2-hydroxyethoxy)methyl counterparts.

The present modification study of **2** indicated that the structure–activity relationships of the 6-benzyl derivatives were similar to those of the aforementioned 6-phenylthio derivatives. The modifications of 6-benzylpyrimidine derivatives at the 1-, 5-, and 6-positions brought about a marked increase in the anti-HIV-1 activity without increasing the cytotoxicity of the compounds.

As shown in Table 2, **33** exhibited activity in peripheral blood lymphocytes (PBLs) against HIV-1 ($HTLV\text{-}III_B$). In addition to $HTLV\text{-}III_B$, an AZT-resistant variant (A012D) of HIV-1 was also highly susceptible to **33**. As previously noted with HEPT,[15] **33** had no effect on the replication of the $LAV\text{-}2_{ROD}$ strain of HIV-2 at its nontoxic concentrations to the host cells.

After studies on pharmacokinetics and following toxicology tests, 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (**33**) was selected as the candidate for clinical trials for AIDS (or ARC) chemotherapy. Pharmacokinetics studies were carried out in rats with **33**.[18] The maximum plasma concentration of **33** was 3.1 μg/mL (10.3 μM) which was reached within 15 min following oral administration at a dose of 50 mg/kg. The oral bioavailability of **33** in rats was 18.4%. From the toxicity studies in rats, the 50% lethal dose (LD_{50}) of **33** was >2000 mg/kg, and **33** did not show any significant toxicity at a dose of 100mg/kg/day for 2 weeks following oral administration. A comparative test of AZT and **33** for their effects on murine bone marrow progenitor cells clearly demonstrated that **33** did not suppress the *in vitro* proliferation of the cells at concentrations up to 5 μM (Table 3). With AZT, however, approximately 50% and 95% inhibition of colony formation was observed at concentrations of 5 and 50 μM, respectively. These results indicate that, unlike AZT, **33** may not cause bone marrow suppression *in vivo*. Synergistic inhibition of HIV-1 replication by **33** and AZT was observed in HIV-1-infected MT-4 cells.[19] Because of its anti-HIV-1 potency, effectiveness against AZT-resistance strains of HIV-1, low toxicity, and synergistic effect with AZT, we believe that **33** seems to be a highly promising candidate for the treatment of AIDS.

Experimental Section

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. ^1H NMR spectra were recorded at 250 MHz on a AC-250 Bruker NMR spectrometer using tetramethylsilane as the internal standard; chemical shifts were recorded in parts per million (ppm). UV spectra were recorded with a Shimadzu UV-260 spectropho-

tometer. Mass spectra were taken on a Hitachi M-80A spectrometer. Silica gel column chromatography was carried out on Merck silica gel 60 H. TLC was performed on silica gel (precoated silica gel plate 60 F254; Merck). Elemental analyses were performed on a Perkin-Elmer 240-C elemental analyzer.

1-[(2-Hydroxyethoxy)methyl]-6-phenylthymine (4). A mixture of **3** (400 mg, 0.91 mmol), Ph_4Sn (1.94 g, 4.54 mmol), and $(Ph_3P)_2PdCl_2$ (70.2 mg, 0.1 mmol) in dioxane (20 mL) was refluxed with stirring under a nitrogen atmosphere for 3 days. The reaction mixture was diluted with EtOH and then filtered. The solution was concentrated to dryness, and the resulting residue was dissolved in 10 mL of THF–AcOH–H_2O (2:2:1, v/v/v). The solution was stirred at room temperature for 14 h and evaporated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$–MeOH, 98:2, v/v) and then crystallized from EtOH to give 74 mg (30%) of **4**: mp 164–166 °C; UV (MeOH) λ_{max} 269 nm (ϵ 11 200); MS *m/z* 276 (M^+); ^{1}H NMR ($CDCl_3$) δ 1.66 (s, 3H, 5-Me), 2.05 (t, J = 6.0 Hz, 1H, HO), 3.53 (t, J = 4.5 Hz, 2H, $HOCH_2CH_2O$), 3.67 (td, J = 4.5, 6.0 Hz, 2H, $HOCH_2CH_2O$), 4.96 (s, 2H, NCH_2O), 7.28–7.52 (m, 5H, Ph), 8.39 (br, 1H, NH). Anal. ($C_{14}H_{14}N_2O_4$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]-6-(phenylethyl)thymine (6). A mixture of **5** (41.2 mg, 0.14 mmol), Pd–C (10%, 5 mg), EtOH (2 mL), dioxane (2 mL), and AcOH (1 mL) was stirred at room temperature for 1 h under 1 atm of hydrogen. The catalyst was removed by filtration and washed with EtOH (2 × 5 mL). The combined filtrates were evaporated to dryness. The residue was crystallized from EtOAc–hexane to give 40.1 mg (94%) of **6**: mp 166 °C; UV (MeOH) λ_{max} 268 nm (ϵ 11 800); MS *m/z* 304 (M^+); ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H, 5-Me), 2.88, 2.99 (A_2B_2, 4H, CH_2CH_2Ph), 3.69–3.81 (m, 4H, OCH_2CH_2O), 5.35 (s, 2H, NCH_2O), 7.15–7.38 (m, 5H, Ph), 8.04 (br, 1H, NH). Anal. ($C_{16}H_{20}N_2O_4 \cdot {}^1/_{10}H_2O$) C, H, N.

General Procedure for the Preparation of 19–22. To a solution of LDA (4.4 mmol) in THF (10 mL) was added 5-ethyl-1-(alkoxymethyl)uracil **7** or **8** (2 mmol) in THF (8 mL) under a nitrogen atmosphere at a rate such that the temperature did not exceed −70 °C. After the mixture had been stirred for 1 h, benzaldehyde or 3,5-dimethylbenzaldehyde (3 mmol) dissolved in THF (5 mL) was added, maintaining the temperature below −70 °C. The mixture was stirred for 1 h below −70 °C and allowed to warm to room temperature. The solution was neutralized with concentrated HCl, poured into brine (20 mL), and extracted with EtOAc (30 mL). The organic layer was washed with saturated $NaHCO_3$ solution (20 mL) and then with brine (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was dissolved in pyridine (20 mL) and added to acetic anhydride (1 mL), and the solution was stirred for 12 h at room temperature. The mixture was poured into saturated $NaHCO_3$ solution (30 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was washed with saturated $NaHCO_3$ solution (3 × 30 mL) and then with brine (3 × 30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$–hexane, 8:2, v/v) and then crystallized from a suitable solvent.

6-(Acetoxyphenylmethyl)-1-[[2-[(*tert*-butyldimethylsilyl)oxy]ethoxy]methyl]-5-ethyluracil (19): yield 71% (from **7**); mp 116–118 °C (EtOH–H_2O); ^{1}H NMR ($CDCl_3$) δ −0.02 (s, 6H, Me_2Si), 0.83 (s, 9H, Me_3C), 0.94 (t, J = 7.5 Hz, 3H, 5-CH_2Me), 2.26 (s, 3H, COMe), 2.46 (q, J = 7.5 Hz, 2H, 5-CH_2Me), 3.52–3.68 (m, 4H, $SiOCH_2CH_2O$), 5.41, 5.49 (ABq, J = 11.1 Hz, 2H, NCH_2O), 7.08 (s, 1H, AcOC*H*), 7.27–7.41 (m, 5H, Ph), 8.45 (br, 1H, NH).

6-[Acetoxy(3,5-dimethylphenyl)methyl]-1-[[2-[(*tert*-butyldimethylsilyl)oxy]ethoxy]methyl]-5-ethyluracil (20): yield 71% (from **7**); mp 133–134 °C (EtOH–H_2O); ^{1}H NMR ($CDCl_3$) δ −0.02 (s, 6H, Me_2Si), 0.83 (s, 9H, Me_3C), 0.98 (t, J = 7.3 Hz, 3H, 5-CH_2Me), 2.31 (s, 3H, COMe), 2.49 (q, J = 7.3 Hz, 2H, 5-CH_2Me), 3.52–3.68 (m, 4H, $SiOCH_2CH_2O$), 5.41 (s, 2H, NCH_2O), 6.82 [s, 2H, Ar-H (2,6)], 6.97 [s, 1H, Ar-H (4)], 7.02 (s, 1H, AcOC*H*), 8.41 (br, 1H, NH).

6-(Acetoxyphenylmethyl)-1-(ethoxymethyl)-5-ethyluracil (21): yield 84% (from **8**); mp 156–157 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 0.93 (t, J = 7.2 Hz, 3H, 5-CH_2Me), 1.04 (t, J = 6.9 Hz, 3H, OCH_2Me), 2.26 (s, 3H, COMe), 2.44 (q, J = 7.2 Hz, 2H, 5-CH_2Me), 3.57 (q, J = 6.9 Hz, 2H, OCH_2Me), 5.39, 5.52 (ABq, J = 10.0 Hz, 2H, NCH_2O), 7.09 (s, 1H, AcOC*H*), 7.28–7.41 (m, 5H, Ph), 9.26 (br, 1H, NH).

6-[Acetoxy(3,5-dimethylphenyl)methyl]-1-(ethoxymethyl)-5-ethyluracil (22): yield 60% (from **8**); mp 148.5 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 0.96 (t, J = 7.5 Hz, 3H, 5-CH_2Me), 1.07 (t, J = 7.1 Hz, 3H, OCH_2Me), 2.25 (s, 3H, COMe), 2.31 (s, 6H, $ArMe_2$), 2.45 (q, J = 7.5 Hz, 2H, 5-CH_2Me), 3.58 (q, J = 7.1 Hz, 2H, OCH_2Me), 5.37, 5.45 (ABq, J = 10.5 Hz, 2H, NCH_2O), 6.84 [s, 2H, Ar–H (2,6)], 6.97 [s, 1H, Ar-H (4)], 7.02 (s, 1H, AcOC*H*), 8.61 (br, 1H, NH).

General Procedure for the Preparation of 23–26. To a solution of LDA (4.4 mmol) in THF (10 mL) was added 5-isopropyl-1-(alkoxymethyl)-2-thiouracil **13** or **14** (2 mmol) in THF (8 mL) under a nitrogen atmosphere at a rate such that the temperature did not exceed −70 °C. After the mixture was stirred for 1 h, benzaldhyde or 3,5-dimethylbenzaldehyde (3 mmol) dissolved in THF (5 mL) was added, maintaining the temperature below −70 °C. The mixture was stirred for 1 h below −70 °C and allowed to warm to room temperature. The solution was neutralized with concentrated HCl, poured into brine (20 mL), and extracted with EtOAc (30 mL). The organic layer was washed with saturated $NaHCO_3$ solution (20 mL) and then with brine (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was dissolved in aqueous 1 N NaOH (20 mL), 35% H_2O_2 (1 mL, 10 mmol) was added, and the solution was stirred at room temperature. After 1 h, the reaction mixture was neutralized with concentrated HCl. The resulting precipitate was collected on a filter and washed with saturated $NaHCO_3$ solution (3 × 30 mL) and H_2O (3 × 30 mL). The precipitate was dried *in vacuo* and dissolved in pyridine (20 mL) and acetic anhydride (1 mL) and the solution stirred for 12 h at room temperature. The mixture was poured into saturated $NaHCO_3$ solution (30 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was washed with saturated $NaHCO_3$ solution (3 × 30 mL) and then with brine (3 × 30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$–hexane, 8:2, v/v) and then crystallized from a suitable solvent.

6-(Acetoxyphenylmethyl)-1-[[2-[(*tert*-butyldimethylsilyl)oxy]ethoxy]methyl]-5-isopropyluracil (23): yield 71% (from **13**); mp 140–141 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ −0.03 (s, 6H, Me_2Si), 0.83 (s, 9H, Me_3C), 0.89 (d, J = 5.5 Hz, 3H, 5-CH*Me*Me′), 1.21 (d, J = 6.5 Hz, 3H, 5-CHMe*Me*′), 2.26 (s, 3H, COMe), 2.88 (qq, J = 5.5, 6.5 Hz, 1H, 5-C*H*MeMe′), 3.51–3.67 (m, 4H, $SiOCH_2CH_2O$), 5.60, 5.70 (ABq, J = 12.2 Hz, 2H, NCH_2O), 7.04 (s, 1H, AcOC*H*), 7.26–7.40 (m, 5H, Ph), 8.36 (br, 1H, NH).

6-[Acetoxy(3,5-dimethylphenyl)methyl]-1-[[2-[(*tert*-butyldimethylsilyl)oxy]ethoxy]methyl]-5-isopropyluracil (24): yield 71% (from **13**); mp 130–131 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ −0.04, −0.03 (s × 2, 3H × 2, Me_2Si), 0.83 (s, 9H, Me_3C), 0.98 (d, J = 5.2 Hz, 3H, 5-CH*Me*Me′), 1.22 (d, J = 6.5 Hz, 3H, 5-CHMe*Me*′), 2.24 (s, 3H, COMe), 2.31 (s, 6H, $ArMe_2$), 2.91 (qq, J = 5.2, 6.5 Hz, 1H, 5-C*H*MeMe′), 3.48–3.68 (m, 4H, $SiOCH_2CH_2O$), 5.58 (s, 3H, NCH_2O), 6.83 [s, 2H, Ar-H (2,6)], 6.97 [s, 1H, Ar-H (4)], 6.99 (s, 1H, AcOC*H*), 8.24 (br, 1H, NH).

6-(Acetoxyphenylmethyl)-1-(ethoxymethyl)-5-isopropyluracil (25): yield 52% (from **14**); mp 175–177 °C (*i*-PrOH); ^{1}H NMR ($CDCl_3$) δ 0.88 (d, J = 6.5 Hz, 3H, 5-CH*Me*Me′), 1.04 (t, J = 7.0 Hz, 3H, OCH_2Me), 1.21 (d, J = 6.9 Hz, 3H, 5-CHMe*Me*′), 2.26 (s, 3H, COMe), 2.88 (qq, J = 6.5, 6.9 Hz, 1H, 5-C*H*MeMe′), 3.61 (q, J = 7.0 Hz, 2H, OCH_2Me), 5.58, 5.70 (ABq, J = 10.9 Hz, 2H, NCH_2O), 7.05 (s, 1H), AcOC*H*), 7.27–7.42 (m, 5H, Ph), 9.06 (br, 1H, NH).

6-[Acetoxy(3,5-dimethylphenyl)methyl]-1-(ethoxymethyl)-5-isopropyluracil (26): yield 47% (from **14**); mp 180 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 0.94 (d, J = 6.3 Hz, 3H, 5-CH*Me*Me′), 1.04 (t, J = 7.1 Hz, 3H, OCH_2Me), 1.22 (d, J = 6.9 Hz, 3H, 5-CHMe*Me*′), 2.24 (s, 3H, COMe), 2.32 (s, 6H, $ArMe_2$), 2.90 (qq, J = 6.3, 6.9 Hz, 1H, 5-C*H*MeMe′), 3.60 (q, J = 7.1 Hz, 2H, OCH_2Me), 5.56, 5.63 (ABq, J = 10.5 Hz, 2H, NCH_2O), 6.85 [s, 2H, Ar-H (2,6)], 6.96 [s, 1H, Ar-H (4)], 6.99 (s, 1H, AcOC*H*), 9.03 (br, 1H, NH).

General Procedure for the Preparation of 27–34. A mixture of 6-(acetoxyarylmethyl)-5-alkyl-1-(alkoxymethyl)-uracil **19–26** (1 mmol) and Pd–C (10%, 50 mg) in AcOH–H_2O–dioxane (20 mL, 2:1:2, v/v/v) was stirred at 60 °C for 15 h under 1 atm of hydrogen. The catalyst was removed by filtration and washed with EtOH (2 × 10 mL). The combined filtrates were concentrated to dryness and crystallized from a suitable solvent.

6-Benzyl-5-ethyl-1-[(2-hydroxyethoxy)methyl]uracil (27): yield 49% (from **19**); mp 121–122 °C (EtOAc–hexane); UV (MeOH) λ_{max} 268 nm (ϵ 11 100); MS *m/z* 304 (M^+); ^1H NMR ($CDCl_3$) δ 1.07 (t, J = 7.4 Hz, 3H, 5-CH_2Me), 1.91 (t, J = 5.7 Hz, 1H, HO), 2.47 (q, J = 7.4 Hz, 2H, 5-CH_2Me), 3.65–3.78 (m, 4H, OCH_2CH_2O), 4.14 (s, 2H, CH_2Ph), 5.17 (s, 2H, NCH_2O), 7.13 [dd, J = 2.0, 6.6 Hz, 2H, Ph-H (2,6)], 7.28–7.39 [m, 3H, Ph-H (3,4,5)], 8.40 [br, 1H, NH). Anal. ($C_{16}H_{20}N_2O_4 \cdot {}^1/_5H_2O$) C, H, N.

6-(3,5-Dimethylbenzyl)-5-ethyl-1-[(2-hydroxyethoxy)-methyl]uracil (28): yield 48% (from **20**); mp 175–177 °C (MeOH–H_2O); UV (MeOH) λ_{max} 268 nm (ϵ 10 700); MS *m/z* 332 (M^+); ^1H NMR ($CDCl_3$) δ 1.08 (t, J = 7.5 Hz, 3H, 5-CH_2Me), 1.93 (t, J = 5.9 Hz, 1H, HO), 2.29 (s, 6H, ArMe_2), 2.47 (q, J = 7.5 Hz, 2H, 5-CH_2Me), 3.65–3.78 (m, 4H, OCH_2CH_2O), 4.06 (s, 2H, CH_2Ar), 5.17 (s, 2H, NCH_2O), 6.70 [s, 2H, Ar-H (2,6)], 6.91 [2, 1H, Ar-H (4)], 8.27 (br, 1H, NH). Anal. ($C_{18}H_{24}N_2O_4 \cdot {}^1/_5H_2O$) C, H, N.

6-Benzyl-1-(ethoxymethyl)-5-ethyluracil (29): yield 88% (from **21**); mp 102–104 °C (EtOH); UV (MeOH) λ_{max} 268 nm (ϵ 10 400); MS *m/z* 288 (M^+); ^1H NMR ($CDCl_3$) δ 1.07 (t, J = 7.4 Hz, 3H, 5-CH_2Me), 1.19 (t, J = 7.0 Hz, 3H, OCH_2Me), 2.47 (q, J = 7.4 Hz, 2H, 5-CH_2Me), 3.60 (q, J = 7.0 Hz, 2H, OCH_2-Me), 4.16 (s, 2H, CH_2Ph), 5.10 (s, 2H, NCH_2O), 7.10–7.39 (m, 5H, Ph), 8.45 (br, 1H, NH). Anal. ($C_{16}H_{20}N_2O_3$) C, H, N.

6-(3,5-Dimethylbenzyl)-1-(ethoxymethyl)-5-ethyluracil (30): yield 85% (from **22**); mp 162–163 °C (EtOH); UV (MeOH) λ_{max} 268 nm (ϵ 10 400); MS *m/z* 316 (M^+); ^1H NMR ($CDCl_3$) δ 1.08 (t, J = 7.5 Hz, 3H, 5-CH_2Me), 1.23 (t, J = 7.0 Hz, 3H, OCH_2Me), 2.28 (s, 6H, ArMe_2), 2.47 (q, J = 7.5 Hz, 2H, 5-CH_2Me), 3.61 (q, J = 7.0 Hz, 2H, OCH_2Me), 4.07 (s, 2H, CH_2Ar), 5.11 (s, 2H, NCH_2O), 6.70 [s, 2H, Ar-H (2,6)], 6.90 [s, 1H, Ar-H (4)], 8.68 (br, 1H, NH). Anal. ($C_{18}H_{24}N_2O_3$) C, H, N.

6-Benzyl-1-[(2-hydroxyethoxy)methyl]-5-isopropyl-uracil (31): yield 54% (from **23**); mp 142–143 °C (EtOH); UV (MeOH) λ_{max} 267 nm (ϵ 10 900); MS *m/z* 318 (M^+); ^1H NMR ($CDCl_3$) δ 1.29 (d, J = 7.0 Hz, 6H, 5-CHMe_2), 2.10 (t, J = 5.2 Hz, 1H, HO), 2.86 (qq, J = 7.0 Hz, 1H, 5-CHMe$_2$), 3.68–3.74 (m, 4H, OCH_2CH_2O), 4.17 (s, 2H, CH_2Ph), 5.19 (s, 2H, NCH_2O), 7.08–7.41 (m, 5H, Ph), 8.87 (br, 1H, NH). Anal. ($C_{17}H_{22}N_2O_4$) C, H, N.

6-(3,5-Dimethylbenzyl)-1-[(2-hydroxyethoxy)methyl]-5-isopropyluracil (32): yield 46% (from **24**); mp 142–143 °C (toluene); UV (MeOH) λ_{max} 267 nm (ϵ 9600); MS *m/z* 346 (M^+); ^1H NMR ($CDCl_3$) δ 1.29 (d, J = 7.2 Hz, 6H, 5-CHMe_2), 1.96 (br, 1H, HO), 2.29 (s, 6H, ArMe_2), 2.85 (qq, J = 7.2 Hz, 1H, 5-CHMe$_2$), 3.69–3.73 (m, 4H, OCH_2CH_2O), 4.08 (s, 2H, CH_2Ar), 5.18 (s, 2H, NCH_2O), 6.71 [s, 2H, Ar-H (2,6)], 6.91 [s, 1H, Ar-H (4)], 8.23 (br, 1H, NH). Anal. ($C_{19}H_{26}N_2O_4$) C, H, N.

6-Benzyl-1-(ethoxymethyl)-5-isopropyluracil (33): yield 85% (from **25**); mp 109–110 °C (EtOH); UV (MeOH) λ_{max} 268 nm (ϵ 10 400); MS *m/z* 302 (M^+); ^1H NMR ($CDCl_3$) δ 1.19 (t, J = 7.0 Hz, 3H, OCH_2Me), 1.29 (d, J = 6.9 Hz, 6H, 5-CHMe_2), 2.87 (qq, J = 6.9 Hz, 1H, 5-CHMe$_2$), 3.62 (q, J = 7.0 Hz, 2H, OCH_2Me), 4.18 (s, 2H, CH_2Ph), 5.12 (s, 2H, NCH_2O), 7.10–7.37 (m, 5H, Ph), 8.40 (br, 1H, NH). Anal. ($C_{17}H_{22}N_2O_3$) C, H, N.

6-(3,5-Dimethylbenzyl)-1-(ethoxymethyl)-5-isopropyluracil (34): yield 69% (from **26**); mp 141–142 °C (EtOH); UV (MeOH) λ_{max} 268 nm (ϵ 11 100); MS *m/z* 330 (M^+); ^1H NMR ($CDCl_3$) δ 1.20 (t, J = 7.0 Hz, 3H, OCH_2Me), 1.30 (d, J = 6.9 Hz, 6H, 5-CHMe_2), 2.28 (s, 6H, ArMe_2), 2.86 (qq, J = 6.9 Hz, 1H, 5-CHMe$_2$), 3.63 (q, J = 7.0 Hz, 2H, OCH_2Me), 4.10 (s, 2H, CH_2Ar), 5.13 (s, 2H, NCH_2O), 6.71 [s, 2H, Ar-H (2,6)], 6.90 [s, 1H, Ar-H (4)], 8.38 (br, 1H, NH). Anal. ($C_{19}H_{26}N_2O_3$), C, H, N.

General Procedure for the Preparation of 35 and 36. A mixture of **29** or **33** (1 mmol), MeCN (5 mL), 2-bromoacetophenone (259 mg, 1.3 mmol), and K_2CO_3 (180 mg, 1.3 mmol) was stirred under reflux for 2 h. The mixture was allowed to cool to room temperature and then poured into brine (10 mL) and extracted with EtOAc (20 mL). The organic layer was washed with aqueous 1 N HCl (20 mL), saturated $NaHCO_3$ solution (20 mL), and then H_2O (20 mL). The organic layer was concentrated to dryness and dissolved in 90% aqueous CF_3COOH (5 mL). The mixture was refluxed for 1 h and then allowed to cool to room temperature and evaporated to dryness. The residue was crystallized from EtOH to give **35** or **36**.

6-Benzyl-5-ethyl-3-phenacyluracil (35): yield 92% (from **29**); mp 221–222 °C (EtOH); ^1H NMR (Me_2SO-d_6) δ 0.83 (t, J = 7.3 Hz, 3H, 5-CH_2Me), 2.31 (q, J = 7.3 Hz, 1H, 5-CH_2Me), 3.83 (s, 3H, CH_2Ph), 5.29 (s, 2H, $COCH_2$), 7.23–7.40 (m, 5H, CH_2Ph), 7.58 [t, J = 7.6 Hz, 2H, COPh-H (3,5)], 7.71 [t, J = 7.6 Hz, 1H, COPh-H (4)], 8.05 [d, J = 7.6 Hz, 2H, COPh-H (2,6)], 11.22 (br, 1H, NH).

6-Benzyl-5-isopropyl-3-phenacyluracil (36): yield 94% (from **33**); mp 212–213 °C (EtOH); ^1H NMR (Me_2SO-d_6) δ 1.06 (d, J = 6.8 Hz, 6H, 5-CHMe_2), 2.89 (qq, J = 6.8 Hz, 1H, 5-CHMe$_2$), 3.85 (s, 2H, CH_2Ph), 5.26 (s, 2H, $COCH_2$), 7.20–7.39 (m, 5H, CH_2Ph), 7.54 [t, J = 7.4 Hz, 2H, COPh-H (3,5)], 7.71 [t, J = 7.4 Hz, 1H, COPh-H (4)], 8.05 [d, J = 7.4 Hz, 2H, COPh-H (2,6)], 11.19 (br, 1H, NH).

General Procedure for the Preparation of 37–40. A mixture of **35** or **36** (1 mmol), Me_2SO (15 mL), BuI (276 mg, 1.5 mmol) or $MeOCH_2CH_2Br$ (209 mg, 1.5 mmol), and K_2CO_3 (207 mg, 1.5 mmol) was stirred at 80 °C for 20 min and then allowed to cool to room temperature. The mixture was evaporated to dryness. The residue was suspended in $CHCl_3$ (20 mL), filtered, and concentrated to dryness. The residue was dissolved in AcOH (10 mL), and Zn powder (3 g) was added. The mixture was stirred under reflux for 14 h and then allowed to cool to room temperature. The mixture was filtered and evaporated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$–hexane, 7:3, v/v) and then crystallized from a suitable solvent to give **37–40**.

6-Benzyl-1-butyl-5-ethyluracil (37): yield 68% (from **35**); mp 172–173 °C (EtOH); UV (MeOH) λ_{max} 275 nm (ϵ 11 100); MS *m/z* 286 (M^+); ^1H NMR ($CDCl_3$) δ 0.87 (t, J = 7.4 Hz, 3H, $CH_2CH_2CH_2Me$), 1.06 (t, J = 7.5 Hz, 3H, 5-CH_2Me), 1.26 (qt, 2H, $CH_2CH_2CH_2$Me), 1.50 (tt, 2H, $CH_2CH_2CH_2$Me), 2.47 (q, J = 7.5 Hz, 2H, 5-CH_2Me), 3.62 (t, J = 8.0 Hz, 2H, CH_2CH_2-CH_2Me), 3.99 (s, 2H, CH_2Ph), 7.09–7.41 (m, 5H, Ph), 8.18 (br, 1H, NH). Anal. ($C_{17}H_{22}N_2O_2$), C, H, N.

6-Benzyl-5-ethyl-1-(2-methoxyethyl)uracil (38): yield 63% (from **35**); mp 112–113 °C (EtOH–H_2O); UV (MeOH) λ_{max} 273 nm (ϵ 11 400); MS *m/z* 288 (M^+); ^1H NMR ($CDCl_3$) δ 1.06 (t, J = 7.5 Hz, 3H, 5-CH_2Me), 2.48 (q, J = 7.5 Hz, 2H, 5-CH_2-Me), 3.33 (s, 3H, OMe), 3.53 (t, J = 5.0 Hz, 2H, OCH_2CH_2N), 3.81 (t, J = 5.0 Hz, 2H, OCH_2CH_2N), 4.18 (s, 2H, CH_2Ph), 7.08–7.41 (m, 5H, Ph), 8.18 (br, 1H, NH). Anal. ($C_{16}H_{20}N_2O_3$) C, H, N.

6-Benzyl-1-butyl-5-isopropyluracil (39): yield 52% (from **36**); mp 142 °C (EtOH); UV (MeOH) λ_{max} 275 nm (ϵ 11 500); MS *m/z* 300 (M^+); ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 7.2 Hz, 3H, $CH_2CH_2CH_2Me$), 1.19–1.34 (m, 8H, 5-CHMe_2, $CH_2CH_2CH_2$Me), 1.54 (tt, 2H, $CH_2CH_2CH_2$Me), 2.86 (qq, J = 7.2 Hz, 1H, 5-CHMe$_2$), 3.63 (t, J = 8.0 Hz, 2H, $CH_2CH_2CH_2$Me), 4.00 (s, 2H, CH_2Ph), 7.08–7.41 (m, 5H, Ph), 8.24 (br, 1H, NH). Anal. ($C_{18}H_{24}N_2O_2$) C, H, N.

6-Benzyl-5-ethyl-1-(2-methoxyethyl)uracil (40): yield 53% (from **35**); mp 105–106 °C ($CHCl_3$–heptane); UV (MeOH) λ_{max} 273 nm (ϵ 10 700); MS *m/z* 302 (M^+); ^1H NMR ($CDCl_3$) δ 1.29 (d, J = 6.9 Hz, 6H, 5-CHMe_2), 2.89 (qq, J = 6.9 Hz, 1H, 5-CHMe$_2$), 3.33 (s, 3H, OMe), 3.55 (t, J = 5.1 Hz, 2H, OCH_2CH_2N), 3.83 (t, J = 5.1 Hz, 2H, OCH_2CH_2N), 4.20 (s, 2H, CH_2Ph), 7.10–7.37 (m, 5H, Ph), 8.52 (br, 1H, NH). Anal. ($C_{17}H_{22}N_2O_3$) C, H, N.

Antiviral Assay Procedures. The activity of the compounds in preventing the replication of HIV-1 (HTLV-III_B strain) and HIV-2 (LAV-2_{ROD} strain) was based on the inhibition of virus-induced cytopathic effect in MT-4 cells as previously described.[16] Briefly, virus stocks were titrated in MT-4 cells and expressed as 50% cell culture infective dose ($CCID_{50}$). MT-4 cells were suspended in culture medium at 1 × 10^5 cells/mL and infected with HIV at a multiplicity of infection (MOI,

ratio of $CCID_{50}$ to cell number) of 0.02. Immediately after virus infection, 100 μL of the cell suspension was brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. The test compounds were dissolved in dimethyl sulfoxide at 50 mM or higher. After a 4 day incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[20] Activity of the compounds against the AZT-resistant clinical isolate of HIV-1 (A012D) was determined by the amount of HIV-1 p24 antigen in the culture supernatant using a sandwich ELISA kit (Abbott) on day 4 after infection of MT-4 cells.

The assay procedure for measuring the anti-HIV-1 activity of the compounds in PBLs was also based on the quantitative detection of HIV-1 p24 antigen in the culture supernatant using a sandwich ELISA kit (Abbott). Phytohemagglutinin-stimulated PBLs (1×10^6 cells/mL) were infected with HIV-1 ($HTLV\text{-}III_B$) at a MOI of 0.2 and cultured at 37 °C in the presence of various concentrations of the test compounds. On day 4 after virus infection, the cells were subcultured at a ratio of 1:5 with fresh culture medium containing appropriate concentrations of the compounds. The assay was performed on day 7 after virus infection.

Cytotoxicity of the compounds was assessed in parallel with their antiviral activity. It was based on the viability of mock-infected MT-4 cells, as monitored by the MTT method.[20]

Colony Formation Assay of Murine Bone Marrow Progenitor Cells.[21] Murine bone marrow cells (2×10^5/mL) were suspended in culture medium containing 10% fetal calf serum (FCS), 0.3% agar, and 50 ng/mL granulocyte macrophage colony stimulating factor (GM-CSF) or interleukin-3 (Il-3). The suspension (1 mL) was brought into each well of a plastic tray and overlayed with culture medium containing FCS, agar, various concentrations of the compounds, and GM-CSF or Il-3. After a 7 day incubation period at 37 °C, the number of colonies was determined.

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