New Uracil Dimers Showing Erythroid Differentiation Inducing Activities

Alessandro Accetta,[†] Roberto Corradini,*[,†] Stefano Sforza,[†] Tullia Tedeschi,[†] Eleonora Brognara,[‡] Monica Borgatti,[‡] Roberto Gambari,*[,‡] and Rosangela Marchelli[†]

Dipartimento di Chimica Organica ed Industriale, Università degli Studi di Parma, Viale G.P. Usberti, 17/A, 43100 Parma, Italy, and BioPhamaNet, Dipartimento di Biochimica e Biologia Molecolare, Sezione di Biologia Molecolare, Università di Ferrara, Via Luigi Borsari, 46- 44100 Ferrara, Italy

Received August 2, 2008

The synthesis of C5 linked uracil dimers was carried out according to a model developed in order to bind adenine in DNA. N1-Alkylated uracil derivatives were synthesized from isoorotic acid (uracil-5-carboxylic acid) or thymine. The carboxylic acid derivatives were condensed with diamines in order to produce dimeric compounds or with monoamines in order to obtain reference monomeric compounds. Some of the derivatives, in particular the uracil dimers, showed antiproliferative and erythroid differentiation induction properties towards human chronic myelogenous leukemia K562 cells, thus indicating that these compounds could represent a new class of drugs useful for the development of antitumor therapy based on the ability to induce terminal differentiation.

Introduction

Pyrimidine derivatives are very important constituents of naturally occurring bioactive compounds, including natural nucleobases and their analogues.[1] A series of pyrimidine derivatives are currently used as drugs; for example, fluorouracil has cytostatic effects and is currently used in cancer therapeutics, and azidothymidine (AZT) was the first applied drug for HIV treatment.

The potential of pyrimidine compounds is linked to the possibility of being used as antagonists in the biosynthetic pathways of pyrimidine nucleobases or in other important processes, by competing for the same binding sites of naturally occurring compounds. For example, oxime libraries based on dimeric uracil derivatives have been proposed for the development of uracil DNA glycosylase (UNG) inhibitors.[2] 5-Substituted uracil derivatives have been described as cytostatic and antiviral compounds.[3]

Another possible use of pyrimidine analogues has recently been explored in connection with the synthesis of tailor-made modified nucleobases to be included in DNA analogues, such as modified oligonucleotides[4,5] or peptide nucleic acids (PNAs).[6,7] For example, extended aromatic analogues of cytosine (named "G-clamp") bearing extra hydrogen bonding sites have been shown to be able to strongly bind guanine by simultaneous formation of Watson−Crick and Hoogsteen base pairing.[8]

The ability to bind to specific sites of DNA is also a characteristic of many bioactive molecules able to act as antibiotics antiproliferative or differentiating drugs. In this respect, several in vitro experimental systems are available for screening purposes. Among them, the K562 cell line, isolated and characterized by Lozzio and Lozzio from a patient with chronic myelogenous leukemia in blast crisis,[9−11] has been proposed as a very useful experimental system to identify (a) antitumor compounds[12,13] and (b) inducers of erythroid differentiation and γ-globin gene expression of possible interest in the therapy of several hematological diseases, including β-thalassemia and sickle cell anemia.[14−20] K562 cells exhibit a low proportion of hemoglobin-synthesizing cells under standard cell growth conditions but are able to undergo erythroid differentiation when treated with a variety of compounds, including short fatty acids,[19] 5-azacytidine,[19] mithramycin and chromomycin,[18,21] cisplatin and cisplatin analogues,[17] tallimustine,[16,19] rapamycin,[22] everolimus,[23] psoralens,[24] and resveratrol.[25] Following erythroid induction, a sharp increase of expression of human ε and γ globin genes is observed in K562 cells, leading to a cytoplasmic accumulation of Hb Portland ($\zeta_2\gamma_2$) and Hb Gower 1 ($\zeta_2\varepsilon_2$).[19] Several antitumor drugs were demonstrated to induce erythroid differentiation of K562 cells. Some of us have recently demonstrated that DNA binding drugs (DBDs[a]) exhibiting antitumor activity are powerful inducers of differentiation of K562 cells, suggesting that the expression of crucial genes involved in terminal erythroid differentiation of these cells is influenced by DBDs.[17,18,21] Several DBDs, such as tallimustine, mithramycin, cisplatin, and angelicin, increase fetal hemoglobin (HbF) production in erythroid precursor cells from normal human subjects.[19] Thus, this experimental cell system appears to be suitable for the screening of molecules able to inhibit cell growth by acting on the activation of terminal differentiation pathways.

In a general project aimed at the synthesis of oligonucleotide analogues, in particular PNA, with modifications able to improve their binding activity,[26−28] we have designed uracil dimers connected with a spacer through the 5-positions (Figure 1). The design was performed by considering as a model the very stable TAT triplet found in PNA/DNA/PNA triplex crystal structure.[29] Since these modified nucleobases could also be considered as potential drugs per se, they were subjected to a screening process for the ability to induce erythroid differentiation and to exhibit cytotoxicity, with positive results for some of the tested compounds. In the present work we describe the synthesis of these uracil dimers and the results obtained in the evaluation of their differentiating properties, which allowed us to define a

* To whom correspondence should be addressed. For R.C.: phone, +39-0521-905406; fax, +39-052-905472; e-mail, roberto.corradini@unipr.it. For R.G.: phone, +39-0532974443; fax, +39-0532-974500; e-mail, gam@unife.it.

[†] Università degli Studi di Parma.

[‡] Università di Ferrara.

[a] Abbreviations: AcCN, acetonitrile; AM1, Austin model 1; DBDs, DNA binding drugs; DCM, dichloromethane; HbF, fetal hemoglobin; DIEA, *N*,*N*-diisopropylethylamine; HMDS, hexamethyldisilazane; TMS-Cl, trimethylsilyl chloride; TFA, trifluoroacetic acid; Tr, trityl; UFF, universal force field.

10.1021/jm800982q CCC: $40.75
Published on Web 12/15/2008

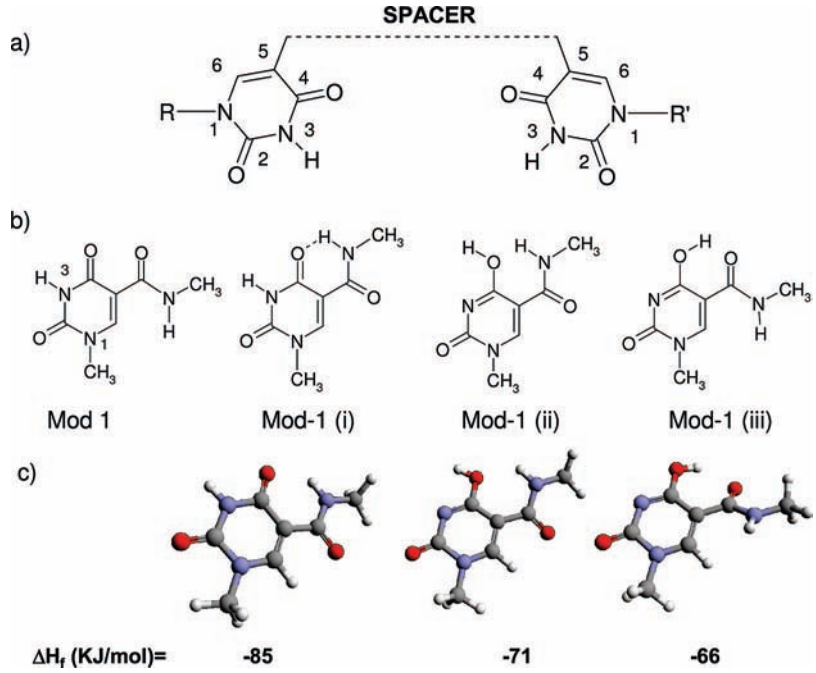

Figure 1. Design of uracil dimers: (a) general scheme of uracil derivatives described in the present study, with numbering given according to uracil nomenclature; (b) tautomeric forms of uracil-5-carboxamide moiety considered for calculations; (c) energy-minimized structures calculated using the AM1 method and corresponding energies in kJ mol^{-1}.

new class of drug candidates, together with a rationale of the possible connection between structure and activity based on their design.

Results and Discussion

Design. The general design of the uracil dimers is reported in Figure 1a. Alkylation of uracil N1 is necessary either to introduce lipophilic groups or for linking this compounds to the backbone of nucleosides, nucleotides, or nucleotide analogues. In all cases the N3 hydrogen was preserved in order to maintain the same hydrogen-bond pairing scheme presented by uracil. In the first part of this project, we designed a series of dimers linked through amide bonds, since molecular modeling (vide infra) indicated that the hydrogen bonding moieties could be preserved using this kind of modification. Since the compounds were designed in order to mimic and reinforce the binding ability of uracil, we checked if the modification introduced was able to induce severe changes in the uracil potential binding sites, since electronegative substituents can shift the tautomeric lactamic−lactimic equilibrium.[30] The most dramatic change in the structure was the introduction of a carboxamide moiety in the 5-position of uracil; thus, we tested the ability of these derivatives to be in the same tautomeric forms, and hence with the same recognition pattern by hydrogen bonds, by means of semiempirical calculation performed on the model compound **Mod-1** (Figure 1b).[31] The structure of **Mod-1** was first minimized by molecular mechanics using universal force field (UFF) and then subjected to energy minimization by a semiempirical method using the Austin Model 1 (AM1). The same procedure was applied to the tautomer **Mod-1(ii)**. The most stable structures obtained in both cases are reported in Figure 1c together with the corresponding enthalpies of formation. The most stable structure **Mod-1(i)** has the amide hydrogen pointing toward the C4 carbonyl oxygen, thus giving rise to an intramolecular hydrogen bond. Both the structures **Mod-1(ii)** and an alternative conformation **Mod-1(iii)** show higher energies. Therefore, the 5-carboxamide compounds are prone to exhibit the same recognition pattern of uracil as originally designed.

Synthesis. In our retrosynthetic design, we used as starting material either isoorotic acid (uracil-5-carboxylic acid) or thymine. As a reference compound, isoorotic acid methyl ester (**1**) was synthesized by reaction with $SOCl_2$ in methanol. Several derivatives were obtained with the purpose of varying the group at N1, the type (monomeric or dimeric) of the amine residue, and the size and rigidity of the spacer, in order to produce chemical diversity.

Direct alkylation of isoorotic acid with reactive substrates, such as methyl or allyl halides, led to dialkylated products at both nitrogen atoms (results not shown); therefore, regioselective monoalkylation of uracil was performed using temporary protection of the carboxylic and carbonyl oxygens with trimethylsilyl groups, through reaction with hexamethyldisilazane (HMDS) in a 3:1 excess and in the presence of trimethylchlorosilane (TMS-Cl) (Figure 2a). This strategy not only allows for monoalkylation, since the positively charged pyrimidinium intermediate prevents further attack, but also directs the alkylation towards the less hindered N1 position. Confirmation of the positions of the substituent in **2** and **3** was obtained by NOE effects between the alkyl group and the CH(6) of uracil as measured by 1D and 2D NOESY spectra.

Reaction of thymine with more hindered long chain primary haloalkanes led to selective monoalkylation at N1 (Figure 2b), thus affording the subtrate **4** suitable for the synthesis of uracil derivatives with a C_8 alkyl chain at N1. Oxidation of the methyl group of **4** with $K_2S_2O_8$ in the presence of copper(II) leads to the uracil-5-carboxaldehyde, which was then oxidized by reaction with sodium chlorite to the corresponding carboxylic acid **5** bearing a C8 alkyl chain at the N1 position.

According to our model, the 2,7-di(aminomethyl)naphthalene **8** was a good candidate as a linking group for allowing cooperative binding to adenine. Therefore, this compound was synthesized using substitution of the corresponding bromide (**6**)

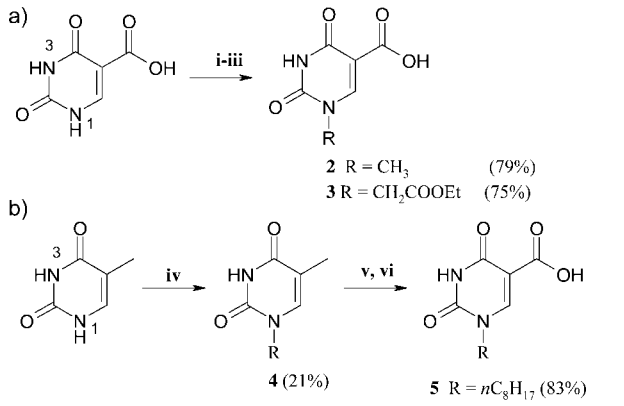

Figure 2. Synthesis of N1-alkylated isoorotic acid derivatives (a) via regioselective alkylation of Isoorotic acid ((i) HMDS,TMS-Cl, reflux 4 h; (ii) CH_3I or ethyl bromoacetate in excess, reflux,18 h; (iii) H_2O/CH_3COOH, room temp, 20 min.) and (b) via oxidation of thymine derivatives ((iv) [Br-*n*-C_8H_{17}, NaH, DMF, 80 °C,4 h]; (v) 2,6-lutidine, $K_2S_2O_8$, $CuSO_4$, H_2O/AcCN, 80 °C, 1,5 h; (vi) $NaClO_2$, NaH_2PO_4; *t*-BuOH/THF, room temp, 24 h).

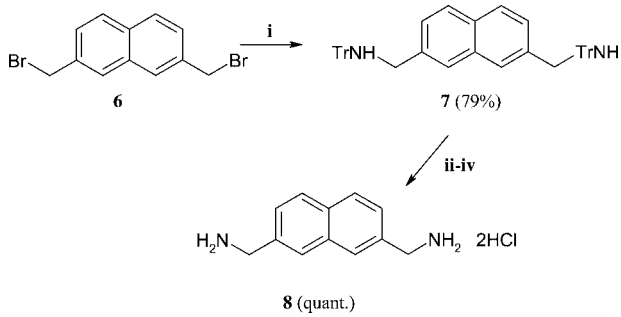

Figure 3. Synthesis of 2,6-dimethylaminonaphthalene: (i) $TrNH_2$, AcCN, 50 °C, 72 h; (ii) TFA, DCM, room temp, 35 min; (iii) MeOH, room temp, 2 h; (iv) 1 M HCl in MeOH.

with tritylamine, followed by acidic solvolysis of the tritylated amine (**7**) (Figure 3).[32]

Several other commercially available diamines were used as linkers for the reaction with the carboxylic derivatives. Butylamine was used for generating reference monomeric compound **10** (Figure 4), and benzylamine was used for generating compound **12**, both containing a uracil moiety with a carboxamide group at C5 (Figure 4).

The first series of derivatives, containing either a methyl or an ethoxycarbonylmethyl group at N1, was synthesized using reaction with thionyl chloride to generate the corresponding acyl chloride, followed by reaction with the corresponding amine in pyridine (Figure 4a).

Since the yields obtained with this method were not optimal (25−30%), mainly because of loss of product during workup, a second series of derivatives was obtained by activation of the carboxylic moiety of **5** with fluoride using 2,4,6-trifluoro-1,3,5-triazine as fluorinating agent, thus providing the stable intermediate **11** which could be isolated. Subsequent reaction of the acyl fluoride **11** with the corresponding diamine or monoamine in acetonitrile gave the compounds **12**−**17** (Figure 4b). Yields were in the range 37−66%, with lowest value for the short, rigid *m*-xylylene bridge.

Screening of Antiproliferative and Differentiation Inducing Properties. We first determined for all the synthesized molecules the effects on cell proliferation. To this aim, K562 cells were cultured in the presence of increasing concentrations of compounds and cell number per milliliter was determined after 3, 4, and 5 days. These time points were selected because between days 3 and 5 untreated control K562 cells are on the log phase of cell growth. A representative experiment is shown in Figure 5, in which the effects on cell growth (panels A and C) and erythroid differentiation (panels B and D) of compounds **9** and **14** are compared. Compound **9** displays very low growth inhibiting properties, while the IC_{50} for compound **14** was about 500 μM. Erythroid differentiation of the compounds under investigation was studied by determining the proportion of benzidine-positive (hemoglobin containing) cells. As clearly evident in Figure 5 (panels B and D), compound **14** was found to stimulate erythroid differentiation while compound **9** was not active (Figure 5, panels A and B). Table 1 indicates the antiproliferative effects (IC_{50} values) and the erythroid induction ability (% of benzidine-positive cells) of all the tested compounds. The best erythroid induction ability was displayed by compound **14**. The data shown in Table 1 were obtained using concentrations of compounds approaching those giving 50% of inhibition of cell growth (these concentrations were chosen to better compare the potential erythroid inducing activity in experimental conditions, leading for most of the compounds tested, to similar effects on cell proliferation rate). In addition, it should be noted that compound **14** was able to induce erythroid differentiation of K562 cells after 6 days of cell culture even if added at concentrations lower than that shown in Table 1 (an average of 52 ± 4.5% of benzidine-positive cells was obtained in four independent experiments with 400 μM compound **14**). Under these experimental conditions no inhibition of cell growth was detectable. In Table 2, the effect of compound **14** is compared with those of other erythroid inducers of K562 cells: the very high induction level suggests that **14** is indeed a very active compound with activity comparable to those of other previously reported compounds for this cell line.

The antiproliferative effect of compound **14** was obtained using other tumor cell lines as cellular targets, including the rhabdomyosarcoma RD[33] and the two breast carcinoma MCF-7[34] and MDA-MB-231[35] cell lines (Table 3). Low antiproliferative activity was found when the human cystic fibrosis bronchial IB3-1 cell line[36,37] was used (Table 3). As far as the effects on K562 cell growth displayed by all the compounds tested (Table 1), some compounds (for instance, compound **1** and **9**) were found to be ineffective in inhibiting cell growth under the experimental conditions employed; all the other compounds were found to be moderately efficient in inhibiting in vitro cell growth of treated K562 cells. As shown for compound **14** (Table 3), the antiproliferative activity of the compounds here studied is not restricted to K562 cells (data not shown). Our results suggest that among the compounds studied some are efficient at inducing erythroid differentiation without exhibiting strong antiproliferative activity (i.e., compound **14**); conversely, some other compounds (i.e., compound **12**) strongly inhibit cell growth of K562 cells without inducing differentiation. When the experiments were conducted at concentration higher than that reported in Table 1, compound **12** was confirmed to be inactive in inducing differentiation (analysis was conducted at 200, 400, and 600 μM without any effects in stimulating the increase of the proportion of benzidine positive K562 cells). Further experiments employing proteomic and transcriptomic analyses are necessary in order to understand the interplay between effects of this class of molecules on cell growth and erythroid differentiation.

Conclusions

In the present paper we have demonstrated for the first time that uracil derivatives bearing a N1-alkyl chain and a 5-car-

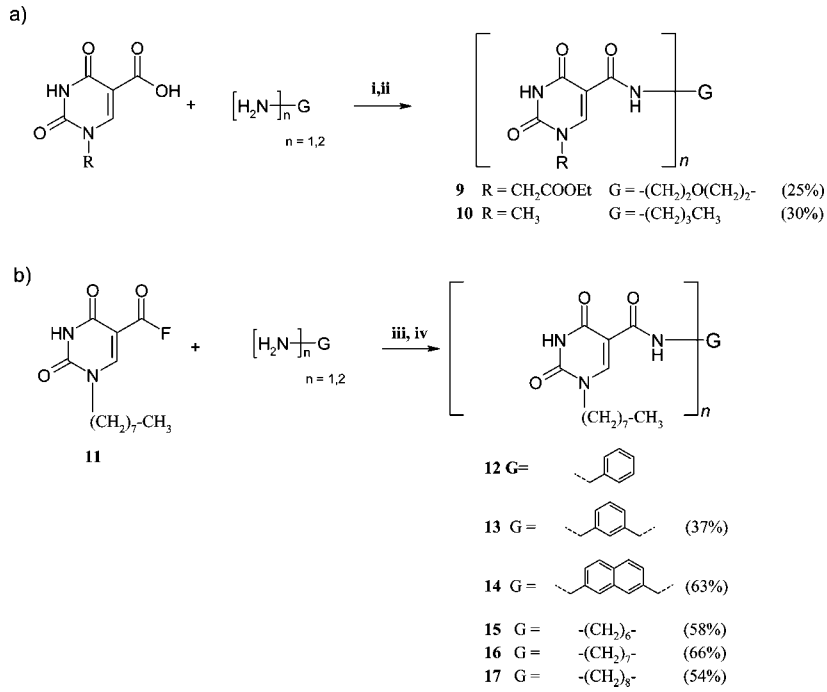

Figure 4. Synthesis of monomeric and dimeric uracil derivatives: (a) **12** and **13** via acyl chloride ((i) $SOCl_2$, DMF, 70−80 °C, 2 h; (ii) $(H_2N)_n$-G, Py, 2 h); (b) **19**−**23** via acyl fluoride ((iii) H_2N-G-NH_2 or H_2N-G-NH_2. 2HCl, DIEA, AcCN, 80 °C, 7 h; (iv) 1 M HCl, 0.5 h).

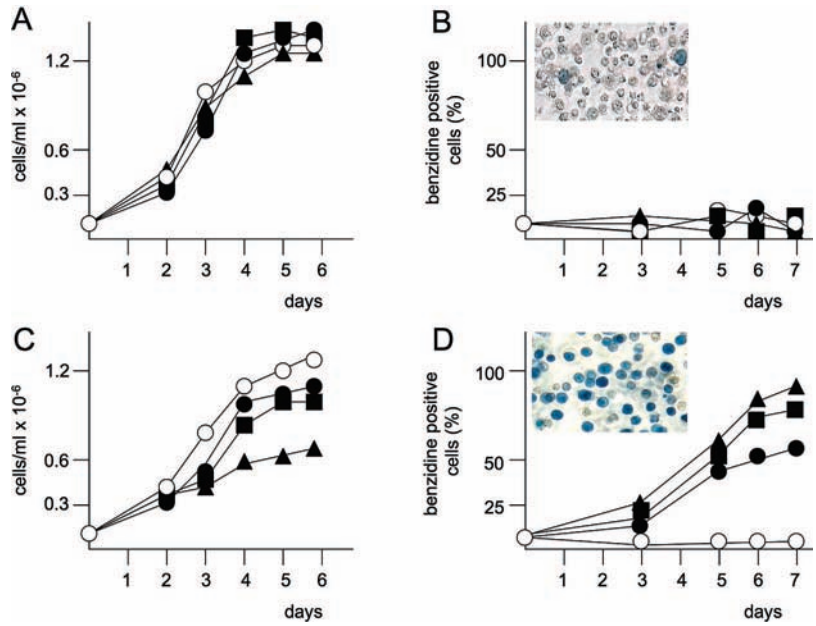

Figure 5. Effects of compounds **9** and **14** on in vitro proliferation (A and C) and erythroid differentiation (B and D) of K562 cells. Cells were cultured in the absence (○) or in the presence of increasing amounts (●, 400 μM; ■, 600 μM; ▲, 800 μM) of compound **9** (A and B) and compound **14** (C and D). After the indicated length of time, cell number per milliliter and the proportion of benzidine-positive cells were determined. Representative images of benzidine staining assay of 6 days of K562 cell cultures treated with compounds **9** and **14** (600 μM) are shown in the relative insets. The results of three independent experiments are reported in Table 1.

boxamide linker can be considered to be a new class of erythroid differentiation inducers, and that dimeric derivatives with suitable spacers have the best performing characteristics: low cytoxicity and higher differentiating ability. The dependence of the induction of increase of hemoglobin-containing cells upon both the length and rigidity of the linking moiety suggests a cooperative mechanism. Furthermore, the best results were obtained with the compound bearing a naphthalene linker, which avoids collapse of the uracil moieties, indicating that a possible recognition of complementary functionalities (such as adenine derivatives) could be implicated in the induction of biological properties. Further studies are needed to evaluate the biological mechanisms implicated in this process.

These findings can be the starting point for the quest for more effective and specific drugs for the induction of terminal erythroid differentiation, ultimately leading to new insights in the treatment of neoplastic diseases with molecules acting by inducing differentiation rather than by exerting cytotoxic effects. In addition, these molecules might be of interest for the experimental treatment of β-thalassemic erythroid cells for which the induction of γ-globin mRNA could be very beneficial.[19,21] In this respect it has been demonstrated that

Table 1. Antiproliferative Effects (IC_{50}) and Percentage of Benzidine-Positive Cells after Treatment with the Various Compounds and Concentration Used[a]

compd	antiproliferative effect IC_{50} (μM)	% of benzidine positive cells after 6 days	concentration (μM)
1	>800	5 ± 3.4	800
5	247 ± 33	30 ± 5.8	300
9	>800	1 ± 0.8	800
10	536 ± 45	5 ± 2.3	800
12	75 ± 7.3	5 ± 3.3	100
13	247 ± 23	40 ± 8.4	200
14	517 ± 63	78 ± 7.3	600
15	600 ± 85	40 ± 5.5	600
16	220 ± 35	50 ± 6.8	600
17	420 ± 93	45 ± 3.5	600

[a] Results are presented as average ± SD of three independent experiments performed. The IC_{50} was calculated as the concentration of compounds necessary to decrease cell number (after 4 days of culture period) at 50% of the values obtained in control untreated K562 cell cultures. The % of benzidine-positive (hemoglobin-containing) cells was determined after 6 days of induction period at concentrations of the tested compounds indicated in the rightmost column.

Table 2. Effects of Compound **14** on in Vitro Growth and Erythroid Differentiation of Human Leukemic K562 Cells

compd	concentration	erythroid induction[a] (% of benzidine-positive cells)
14	600 μM	78 ± 7.3
Ara-C	500 nM	78.3 ± 4.5
mithramycin	100 nM	86.8 ± 8.3
rapamycin	1.0 mM	75.5 ± 7.5
butyric acid	2.0 mM	32.5 ± 3.4

[a] Results are presented as average ± SD (three independent experiments performed) of the % benzidine-positive (hemoglobin-containing) cells after 6 days of induction period at the indicated concentrations of the tested compounds.

Table 3. Effects of Compound **14** on in Vitro Growth of Human Cell Lines

cell line	phenotype/origin	antiproliferative effect[a] (IC_{50}, μM)
K562	chronic myelogenous leukemia (CML)	517 ± 63
RD	rhabdomyosarcoma	76.6 ± 9.3
MDA	breast cancer	171.2 ± 27.5
MCF7	breast cancer	197.4 ± 32.4
IB3-1	bronchial epithelial cell line (cystic fibrosis)	505 ± 58

[a] Results are presented as average ± SD (three independent experiments performed) of concentration needed to obtain 50% inhibition of cell growth after a 4 day culture period.

inducers of K562 erythroid differentiation are often able to induce fetal hemoglobin production in erythroid cells isolated from β-thalassemia patients.[19]

Experimental Section

Chemicals were purchased from Aldrich, Fluka, Acros, or Merck and have been used without further purification. TLC was performed on aluminum sheets coated with silca gel F_{254}, 0.2 mm. Flash chromatography was carried out under nitrogen pressure using Merck silica gel 60H. NMR spectra were measured on Brucker AC 300 and on a Varian INOVA 600 instruments. Melting points were determined on Gallenkamp melting apparatus and are uncorrected. FT-IR spectra were recorded on Nicolet FT-IR. Mass spectra were recorded on Waters Acquity mass spectrometer.

Molecular Mechanics Calculations. Starting structures were generated using the sketch module of ArgusLAB. Geometry optimization was carried out using UFF (universal force field). Electrostatic terms were treated with simple cutoff (10−8 Å), and structures were optimized using the BFGS (Broyden−Fletcher−Golfarb-Shanno) algorithm.

Semiempirical Calculations. Geometry optimization was carried out using the AM1 (NDDO) module of ArgusLAB, the BFGS algorithm with SCF convergence of 1.5936×10^{-13} au for energy, and the STO 3G as the basis set.[31]

Synthetic Procedures. Methyl 2,4-Dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1). A round-bottomed flask containing 50 mL of MeOH was cooled at 10 °C, and $SOCl_2$ (9.62 mmol, 0.70 mL) was added carefully. After 5 min isoorotic acid was added and the mixture was heated and refluxed for 18 h. Then the solvent was removed, and the white powder obtained was washed many times with MeOH, suspended in hexane, and filtered to yield 1.10 g of **1** (yield >99%). Dec T > 250 °C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ (ppm) 3.68 (s, 3H), 8.13 (s, 1H), 11.31 (s, 1H), 11.60 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ (ppm) 50.6, 99.8, 155.2, 157.1, 161.7, 164.5. FT-IR (KBr pellet) ν (cm^{-1}) 1781, 1738, 1620. MS (ESI+): m/z [MH$^+$] 171.0, [MNa$^+$] 192.9. Anal. ($C_6H_6N_2O_4$) H. C: calcd, 42.36; found, 41.77. N: calcd, 16.47; found, 15.64.

1-Methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic Acid (2). Isoorotic acid (1.83 mmol, 300 mg) was suspended in hexamethydisilazane (HMDS) (11.50 mmol, 2.5 mL), and trimethylchlorosilane (TMS-Cl) (1.10 mmol, 0.10 mL) was added. The mixture was refluxed in a closed tube at 120 °C for 4 h (until the mixture appeared colorless). The reaction temperature was raised to room temperature, and then iodomethane (30.0 mmol, 1.85 mL) was added. The mixture was then heated to 50 °C and kept overnight at the same temperature. The solvent was then evaporated, and the residue was stirred with 3 mL of ice water and 3 mL of glacial acetic acid for 20 min. The precipitate formed was collected by filtration and washed with cold water and ethyl acetate to afford 246 mg of a pale-yellow solid corresponding to the product (yield = 79%). Mp 258.6−259.2 °C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ (ppm) 3.38 (s, 3H), 8.67 (s, 1H), 12.19 (s, 1H), 12.67 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ (ppm) 36.2, 100.7, 150.0, 154.2, 163.3, 164.8. FT-IR (KBr pellet) ν (cm^{-1}) 1752, 1728, 1704, 1618. MS (ESI+): m/z [MH$^+$] 170.0, [MK$^+$] 209.5. Anal. ($C_6H_6N_2O_4$) N. C: calcd, 42.36; found, 39.61. H: calcd, 3.55; found, 4.01.

1-(2-Ethoxy-2-oxoethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic Acid (3). Compound **3** was synthesized as described above for compound **2**, using ethyl bromoacetate as alkylating agent (yield = 75%). Mp 184.2−187.2 °C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ (ppm) 1.21 (t, 3H, J = 7.3 Hz), 4.16 (q, 2H, J = 7.3 Hz), 4.69 (s, 2H), 8.71 (s, 1H), 12.24 (s, 1H), 12.67 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ (ppm) 13.8, 49.2, 61.3, 102.2, 149.7, 153.5, 163.1, 163.3, 167.4. FT-IR (KBr pellet) ν (cm^{-1}) 1793, 1740, 1712, 1625. MS (ESI+): m/z [MH$^+$] 243.0, [MNa$^+$] 265.0. Anal. ($C_9H_{10}N_2O_6$) C: calcd, 44.63; found, 43.91. H: calcd, 4.16; found, 4.90. N: calcd, 11.57; found, 10.54.

5-Methyl-1-octylpyrimidine-2,4(1*H*,3*H*)-dione (4). Compound **4** was synthesized according to a literature procedure.[38] Mp 111.9−113.5 °C (yield = 22%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) δ (ppm) 0.82 (t, 3H, J = 6.9 Hz), 1.21−1.25 (m, 10H), 1.61 (q, 2H, J = 6.5 Hz), 3.64 (t, 2H, J = 6.5 Hz), 6.96 (s, 1H), 10.09 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C) δ (ppm) 12.2, 13.9, 22.5, 26.3, 29.0, 29.1, 31.6, 48.4, 110.4, 140.4, 151.1, 164.8. FT-IR (KBr pellet) ν (cm^{-1}) 3160, 3067, 3029, 2955, 2926, 2854, 1692, 1653. MS (ESI+): m/z [MH$^+$] 239.2, [MNa$^+$] 261.3, [MK$^+$] 277.2. Anal. ($C_{13}H_{22}N_2O_2$) C, H, N.

1,2,3,4-Tetrahydro-1-octyl-2,4-dioxopyrimidine-5-carboxylic Acid (5). A solution of **4** (2.55 g, 10.2 mmol) and 2,6-lutidine (4.3 mL) in acetonitrile (40 mL) was added to a stirring solution of $K_2S_2O_8$ (5.75 g, 21 mmol) and $CuSO_4$ (0.66 g, 4.2 mmol) in water (40 mL). The mixture was heated at 80 °C for 2 h. Then the mixture was dried, and the residue was partitioned between ethyl acetate and saturated aqueous solution of EDTA. The ethyl acetate was removed from the organic extract, and the resulting yellow oil was dissolved in 100 mL of *t*-BuOH−THF−*i*-butene (6:3:1) mixture. To this mixture a solution of $NaClO_2$ (12 g, 105 mmol) and $Na_2H_2PO_4$ monohydrate (7.5 g, 52.5 mmoles) in water (25 mL) was added dropwise over a period of 30 min. Then the mixture was stirred at room temperature overnight. The solvent was removed and the residue partitioned between saturated aqueous $KHSO_4$ and

ethyl acetate. The organic phase was evaporated, and the residue was partitioned between methylene chloride and aqueous NaOH (2 M). The aqueous extract was neutralized with concentrated HCl at pH 3, and a pale-yellow solid precipitated. The solid was collected by filtration, washed with abundant cold water, and recrystallized from acetone−water to obtain 1.54 g (83%) of desired product. Mp 127.2−128.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) δ (ppm) 0.88 (t, 3H, J = 6.9 Hz), 1.26−1.33 (m, 10H), 1.70−1.77 (m, 2H), 3.88 (t, 2H, J = 7.7 Hz), 8.46 (s, 1H), 9.40 (s, 1H), 12.16 (s-broad, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C) δ (ppm) 14.0, 22.5, 26.2, 28.9, 31.6, 50.5, 102.0, 149.0, 152.7, 162.9, 165.0. IR (KBr pellet) ν (cm^{-1}) 3461, 3420, 3179, 3056, 2956, 2924, 2855, 1744, 1701, 1668. MS (ESI+): m/z [MNa^+] 291.2. Anal. ($C_{13}H_{20}N_2O_4$) C, H, N.

***N,N′*-[Naphthalene-2,7-diyldi(methylene)]bis(1,1,1-triphenylmethanamine) (7).** 2,7-Dibromonaphthalene (1.92 mmol, 610 mg) and tritylamine (7.71 mmol, 2.0 g) were dissolved in 30 mL of acetonitrile and heated at 50 °C for 96 h. Then the solvent was removed and the residue was partitioned between saturated $NaHCO_3$ and DCM. The organic layer was dried with Na_2SO_4 and filtered, and the solvent was evaporated. The solid obtained was purified by flash chromatography on silica gel using a hexane−dichloromethane 6/4 mixture to afford 990 mg of **7** as a white solid (yield = 79%). Mp 221.6−223.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) δ (ppm) 1.28 (s, 2H), 3.51 (s, 4H), 7.21−7.26 (m, 6H), 7.31−7.36 (m, 12H), 7.46 (dd, 2H, J_1 = 8.4 Hz, J_2 = 1.3 Hz), 7.61−7.64 (m, 12H), 7.79 (d, 2H, J = 8.4 Hz), 7.88 (d, 2H, J = 1.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C) δ (ppm) 48.0, 71.0, 125.7, 126.2, 126.3, 126.3, 127.6, 127.9, 128.6, 131.7, 133.5, 138.6, 146.0. FT-IR (KBr pellet) ν (cm^{-1}) 3315, 3057, 3019, 2850, 1488, 1448. MS (ESI+): m/z [M-2Tr-NH_3^+] 170.1, [Tr^+] 243.2. Anal. ($C_{50}H_{42}N_2$) H, N. C: calcd, 89.51; found, 88.85.

Naphthalene-2,7-diyldimethanamine Hydrochloride (8). Compound **7** (1.48 mmol, 960 mg) was dissolved in a mixture of 10 mL of TFA and 7 mL of DCM (an intense yellow color appears immediately after the addition of TFA), and the mixture was stirred at room temperature for 40 min. Then 10 mL of MeOH was added and the mixture was stirred for 1 h until it became colorless. The solvent mixture obtained was evaporated, and the resulting oil was dissolved in a minimum amount of 2 M HCl in MeOH and stored at −20 °C for 1 h. A white precipitate appeared, which was separated by centrifugation and washed with diethyl ether to afford 365 mg of the corresponding diamine hydrochloride (yield = 96%). Dec T > 285 °C. ^{1}H NMR (300 MHz, D_2O, 25 °C) δ (ppm) 4.33 (s, 4H), 7.59 (dd, 2H, J_1 = 8.5 Hz, J_2 = 1.7 Hz), 7.98 (d, 2H, J = 1.7 Hz), 8.02 (d, 2H, J = 8.4 Hz). ^{13}C NMR (75 MHz, D_2O, 25 °C) δ (ppm) 46.5, 130.2, 131.6, 132.3, 134.5, 136.1, 136.2. FT-IR (KBr pellet) ν (cm^{-1}) 3125, 3033, 3013, 2997, 2965, 2867, 1599, 1495, 1480. MS (ESI+): m/z [$MH^+ - NH_3$] 170.0, [MH^+] 187.1. Anal. (free amine) ($C_{12}H_{14}N_2$) H, N. C: calcd, 77.38; found, 76.77.

Diethyl 2,2′-{Oxybis[ethane-2,1-diyliminocarbonyl(2,4-dioxo-3,4-dihydropyrimidine-5,1-diyl)]}diacetate (9). Dry DMF (3 mL), compound **3** (0.88 mmol, 150 mg), and $SOCl_2$ (1.80 mmol, 130 μL) were heated to 80 °C for 2 h under N_2 atmosphere. Then 2-(aminoethoxy)ethanamine (0.44 mmol, 45 mg) and 3 mL of dry pyridine were added. After 2 h the solvent was removed under reduced pressure and the resulting oil was suspended in 10 mL of water. A yellow, pale precipitate appeared and was collected and washed with methanol and ethyl ether to afford 45 mg of **9** (yield = 25%). Dec T > 285 °C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ (ppm) 3.35 (s, 6H), 3.41 (q, 4H, J = 6.0 Hz), 3.50 (t, 4H, J = 6.0 Hz), 8.45 (s, 2H), 8.84 (t, 2H, J = 6.0 Hz), 11.77 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ (ppm) 35.9, 38.2, 67.7, 104.0, 150.3, 151.6, 161.7, 163.5. FT-IR (KBr pellet) ν (cm^{-1}) 1730, 1696, 1611, 1636. MS (ESI+): m/z [MH^+] 409.1, [MNa^+] 431.1. Anal. ($C_{22}H_{28}N_6O_{11}$) H. C: calcd, 47.83; found, 45.92. N: calcd, 15.21; found, 14.79.

***N*-Butyl-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (10).** Dry DMF (3 mL), compound **2** (2.35 mmol, 400 mg), and $SOCl_2$ (4.70 mmol, 341 μL) were heated to 80 °C for 2 h under N_2 atmosphere, and then butylamine (4.70 mmol, 470 μL) and 6 mL of dry pyridine were added. After 2 h the solvent was removed under reduced pressure and the resulting yellow oil was suspended in 4 mL of water and acidified to pH 2. The precipitate collected by filtration was washed with NaOH (1 M), abundant water, MeOH, and diethyl ether to afford 157 mg of a **10** as a white solid (yield = 30%). Mp 244.2−245.2 °C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ (ppm) 0.88 (t, 3H, J = 7.2 Hz), 1.29 (m, 2H, J = 6.9 Hz), 1.45 (m, 2H, J = 6.9 Hz), 3.25 (q, 2H, J = 6.3 Hz), 3.36 (s, 3H), 8.46 (s, 1H), 8.72 (t, 1H, J = 5.6 Hz), 11.80 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ (ppm) 13.5, 19.4, 31.1, 35.9, 37.8, 104.1, 150.3, 151.6, 161.5, 163.7. FT-IR (KBr pellet) ν (cm^{-1}) 3441, 3306, 3170, 3047, 2951, 2874, 2835, 1738, 1683, 1615. MS (ESI+): m/z [MH^+] 226.2. Anal. ($C_{10}H_{15}N_3O_3$) C, H, N.

1,2,3,4-Tetrahydro-1-octyl-2,4-dioxopyrimidine-5-carbonyl Fluoride (11). To a solution of compound **5** (1.5 g, 5.57 mmol) in 30 mL of dry dichloromethane, cyanuric fluoride (2.9 mL, 33.42 mmol) was added dropwise, followed by addition of 0.6 mL of dry pyridine. The mixture was kept under argon and stirred at room temperature overnight. The reaction mixture was extracted with water, and the organic layer was dried over Na_2SO_4 and filtered on a sintered glass (G5) funnel. The solvent was removed to yield a yellow-brown solid. Recrystallization from chloroform−hexane afforded 1.12 g (75%) of **11**. Mp 125.6−127.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) δ (ppm) 0.87 (t, 3H, J = 6.9 Hz), 1.27−1.39 (m, 10H), 1.72−1.76 (m, 2H), 3.86 (t, 2H, J = 7.2 Hz), 8.28 (s,1H), 9.14 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C) δ (ppm) 14.0, 22.5, 26.2, 28.9, 29.0, 29.6, 31.6, 50.5, 99.5 (d, $^2J_{CF}$ = 61.6 Hz), 149.0, 152.1 (d, $^1J_{CF}$ = 333.0 Hz), 154.6, 158.2. FT-IR (KBr pellet) ν (cm^{-1}) 3447, 3182, 3053, 2959, 2923, 2854, 1840, 1820, 1800, 1726, 1696, 1619. MS (ESI+): m/z [MNa^+] 293.2, [MK^+] 309.2. Anal. ($C_{13}H_{19}FN_2O_3$) H. C: calcd, 57.77; found, 56.85. N: calcd, 10.36; found, 11.37.

General Procedure for Compounds 12−17. A solution of **11** (0.37 mmol) and DIEA (1.85 mmol) in acetonitrile (6 mL) was stirred at room temperature for 5 min. Then the diamine or the corresponding hydrochloride (0.185 mmoles) was added and the mixture was refluxed for 7 h. The reaction mixture was poured into 20 mL of 1 M aqueous HCl, and a yellow-brown precipitate appeared. The solid was collected by filtration and washed with water to afford the product.

***N*-Benzyl-1-octyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (12).** Compound **12** was synthesized according the general procedure described above using an 10-fold excess of benzylamine (yield = 88%). Mp 180.3−181.6 °C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ (ppm) 0.85 (t, 3H, J = 6.3 Hz), 1.21−1.29 (m, 10H), 1.56−1.60 (m, 2H), 3.80 (t, 2H, J = 7.2 Hz), 4.48 (d, 2H, J = 6.0 Hz), 7.24−7.35 (m, 5H), 8.50 (s, 1H), 9.13 (t, 1H, J = 6.0 Hz), 11.83 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ (ppm) 13.9, 22.0, 25.6, 28.3, 28.5, 31.1, 42.0, 48.4, 99.5, 104.4, 126.8, 127.2, 128.3, 139.2, 150.0, 151.0, 161.7, 163.5. FT-IR (KBr pellet) ν (cm^{-1}) 3302, 3169, 3115, 3045, 2955, 2922, 2853, 1727, 1678, 1632, 1607. MS (ESI+): m/z [MH^+] 358.3, [M_2H^+] 715.6. Anal. ($C_{20}H_{27}N_3O_3$) H, N. C: calcd, 67.20; found, 65.47.

***N,N′*-Phenylene-1,3-diylbismethylene(1-octyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide) (13).** Yield = 37%. Dec T > 210 °C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ (ppm) 0.85 (t, 6H, J = 6.6 Hz), 1.19−1.30 (m, 20H), 1.55−1.64 (m, 4H), 3.81 (t, 4H, J = 7.5 Hz), 4.46 (d, 2H, 6.0 Hz), 7.16−7.31 (m, 4H), 8.50 (s, 2H), 9.12 (t, 2H, J = 6 Hz), 11.84 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ (ppm) 13.9, 22.0, 25.6, 28.3, 28.5, 31.1, 42.0, 48.42, 104.3, 125.7, 128.5, 139.3, 149.7, 151.1, 153.4, 161.7, 163.1. FT-IR (KBr pellet) ν (cm^{-1}) 3299, 3235, 3015, 2956, 2922, 2852, 2813, 1731, 1693, 1637, 1608. HRMS (MALDI+): m/z [$M + Na^+$] calcd for $C_{34}H_{48}N_6NaO_6^+$, 659.3533; found 659.3961. Anal. ($C_{34}H_{48}N_6O_6$) H, N. C: calcd, 64.13; found, 62.42.

***N,N′*-Naphthalene-2,7-diylbismethylene(1-octyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide) (14).** Yield = 66%). Dec T > 280 °C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ (ppm) 0.85 (t, 6H, J = 6.9 Hz), 1.17−1.30 (m, 20H), 1.55−1.64 (m, 4H), 3.81 (t, 4H, J = 6.6 Hz), 4.64 (d, 2H, 5.4 Hz), 7.42 (d, 2H, J = 8.4 Hz),

7.71 (s, 2H), 7.85 (d, 2H, $J = 8.4$ Hz), 9.22 (t, 2H $J = 5.4$ Hz), 11.85 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6, 90 °C) δ (ppm) 13.1, 21.3, 25.1, 27.77, 27.79, 27.84, 41.9, 48.0, 104.4, 124.9, 125.2, 127.2, 130.9, 132.5, 136.6, 150.3, 157.6, 158.9, 161.4. FT-IR (KBr pellet) ν (cm^{-1}) 3297, 3128, 3010, 2956, 2920, 2851, 2809, 1728, 1696, 1636, 1608. HRMS (MALDI+): *m/z* [M + Na$^+$] calcd for $C_{38}H_{50}N_6NaO_6$ 709.3690, found 709.4341. Anal. ($C_{38}H_{50}N_6O_6 \cdot H_2O$) C, H, N.

***N,N′*-Hexane-1,6-diylbis(1-octyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide) (15).** Yield = 58%. Dec $T > 237$ °C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ (ppm) 0.84 (t, 6H, $J = 6.5$ Hz), 1.20−1.28 (m, 24H), 1.42−1.49 (m, 4H), 1.54−1.61 (m, 4H), 3.25 (m, 4H, $J = 5.7$ Hz), 3.80 (t, 4H, $J = 7.1$ Hz), 8.45 (s, 2H), 8.74 (t, 2H, $J = 5.7$ Hz), 11.83 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ (ppm) 13.8, 21.9, 25.5, 25.9, 28.2, 28.4, 28.8, 28.9, 31.0, 38.1, 48.3, 104.4, 149.9, 150.6, 161.4, 163.5. FT-IR (KBr pellet) ν (cm^{-1}) 3298, 3241, 3013, 2956, 2921, 2853, 2813, 1729, 1697, 1635, 1608. HRMS (ESI+): *m/z* [M + Na$^+$] calcd for $C_{32}H_{52}N_6NaO_6^+$, 639.3846; found, 639.3835. Anal. ($C_{32}H_{52}N_6O_6$) H, N. C: calcd, 62.31; found, 61.28.

***N,N′*-Heptane-1,7-diylbis(1-octyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide) (16).** Yield = 66%. Mp 226.4−229.2 °C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ (ppm) 0.85 (t, 6H, $J = 6.8$ Hz), 1.21−1.29 (m, 26H), 1.42−1.48 (m, 4H), 1.54 − 1.60 (m, 4H), 3.24 (m, 4H, $J = 6.4$ Hz), 3.79 (t, 4H, $J = 7.1$ Hz), 8.45 (s, 2H), 8.73 (t, 2H, $J = 6.4$ Hz), 11.82 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ (ppm) 13.8, 21.9, 25.5, 26.2, 28.2, 28.4, 28.9, 31.0, 38.1, 104.4, 149.9, 150.6, 161.4, 163.5. FT-IR (KBr pellet) ν (cm^{-1}) 3296, 3135, 3011, 2956, 2921, 2853, 2814, 1727, 1697, 1636, 1608. HRMS (ESI+): *m/z* [M + Na$^+$] calcd for $C_{33}H_{54}N_6NaO_6^+$, 653.4003; found, 653.4008. Anal. ($C_{33}H_{54}N_6O_6$) calcd, 62.83; found, 61.54; C, H, N.

***N,N′*-Octane-1,8-diylbis(1-octyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide) (17).** Yield = 54%. Mp 225.2−227.8 °C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ (ppm) 0.84 (t, 6H, $J = 6.8$ Hz), 1.21−1.28 (m, 28H), 1.42−1.50 (m, 4H), 1.54−1.60 (m, 4H), 3.23 (m, 4H, $J = 6.1$ Hz), 3.80 (t, 4H, $J = 6.8$ Hz), 8.45 (s, 2H), 8.73 (t, 2H, $J = 6.4$ Hz), 11.82 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ (ppm) 13.9, 22.0, 25.6, 26.3, 28.3, 28.5, 28.7, 29.0, 29.1, 31.1, 38.2, 48.4, 104.5, 150.0, 150.7, 161.5, 163.6. FT-IR (KBr pellet) ν (cm^{-1}) 3299, 3132, 3013, 2956, 2919, 2851, 2813, 1731, 1696, 1635, 1608. HRMS (ESI+): *m/z* [M + Na$^+$] calcd for $C_{34}H_{56}N_6NaO_6^+$, 667.4159; found, 667.4171. Anal. ($C_{32}H_{52}N_6O_6$) H, N. C: calcd, 63.33; found, 62.11.

Biological Activity. The human chronic myelogenous leukemia K562 (9), rhabdomyosarcoma RD,[33] breast cancer MCF7[34] and MDA-MB-231,[35] and cystic fibrosis bronchial IB3-1[36,37] were maintained in a humidified atmosphere of 5% CO_2/air in RPMI 1640 medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS; Celbio, MI, Italy), 50 units/mL penicillin, and 50 μg/mL streptomycin.[19] In order to determine the ability of the tested compounds to inhibit cell growth and to induce erythroid differentiation, K562 cells (30 000 cells/mL) were cultured in the absence or in the presence of the indicated concentrations of compounds and the cell number per milliliter was determined with a ZF Coulter counter (Counter Electronics, Hialeah, FL) at different days from the culture setup. In order to verify possible effects on erythroid differentiation, the proportion of benzidine-positive K562 cells was determined and compared to the values obtained employing other known inducers of erythroid differentiation, including cytosine arabinoside (Ara-C),[19] mithramycin,[21] rapamycin,[22] and butyric acid.[19]

Acknowledgment. This work was partially supported by a grant from MIUR (PRIN2005 Grant No. 2005038704). R.G. is supported by Associazione Italiana Ricerca sul Cancro (AIRC), Fondazione Cariparo (Cassa di Risparmio di Padova e Rovigo), Associazione Veneta per la Lotta alla Talassemia, Rovigo, Italy (AVLT), Telethon (Grant GGP07257), and STAMINA Project. We thank the EU Project ITHANET (eInfrasctructure for Thalassemia Research Network) for support. Valentina Mileo (Chiesi Farmaceutici) is gratefully acknowledged for performing the HRMS experiments.

Supporting Information Available: Full combustion data analysis for compounds **1**−**5** and **7**−**17**, 1D NOESY of N1 substituted compound **3**, and graphs of cell proliferation and differentiation for compound **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Lagoja, I. M. Pyrimidine as costituent of natural biologically active compounds. *Chem. Biodiversity* **2005**, *2*, 1–50.
(2) Jiang, Y. L.; Krosky, D. J.; Seiple, L.; Stivers, J. T. Uracil-directed ligand tethering: an efficient strategy for uracil DNA glycosylase (UNG) inhibitor development. *J. Am. Chem. Soc.* **2005**, *127*, 17412–17420.
(3) Gazivoda, T.; Raić-Malić, S.; Marjanović, M.; Kralj, M.; Pavelić, K.; Balzarini, J.; De Clercq, E.; Mintas, M. The novel C-5 aryl, alkenyl, and alkynyl substituted uracil derivatives of L-ascorbic acid: synthesis, cytostatic, and antiviral activity evaluations. *Bioorg. Med. Chem.* **2007**, *15*, 749–758.
(4) Benner, S. A. Understanding nucleic acids using synthetic chemistry. *Acc. Chem. Res.* **2004**, *37*, 784–797.
(5) Krueger, A. T.; Lu, H.; Lee, A. H. F.; Kool, E. T. Synthesis and properties of size-expanded DNAs: toward designed, functional genetic systems. *Acc. Chem. Res.* **2007**, *40*, 141–150.
(6) Nielsen, P. E. Peptide nucleic acids: on the road to new gene therapeutic drugs. *Pharmacol. Toxicol.* **2000**, *86*, 3–7.
(7) Wojciechowski, F.; Hudson, R. H. E. Nucleobase modifications in peptide nucleic acids. *Curr. Top. Med. Chem.* **2007**, *7*, 667–679.
(8) Flanagan, W. M.; Wolf, J. J.; Olson, P.; Grant, D.; Lin, K. Y.; Wagner, R. W.; Matteucci, M. D. A cytosine analog that confers enhanced potency to antisense oligonucleotides. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3513–3518.
(9) Lozzio, C. B.; Lozzio, B. B. Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. *Blood* **1975**, *45*, 321–334.
(10) Rutherford, T.; Clegg, J. B.; Higgs, D. R.; Jones, R. W.; Thompson, J.; Weatherall, D. J. Embryonic erythroid differentiation in the human leukemic cell line K562. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 348–352.
(11) Rutherford, T. R.; Clegg, J. B.; Weatherall, D. J. K562 human leukemic cells synthesize embryonic hemoglobin in response to hemin. *Nature* **1979**, *280*, 164–165.
(12) Lampronti, I.; Bianchi, N.; Zuccato, C.; Medici, A.; Bergamini, P.; Gambari, R. Effects on erythroid differentiation of platinum(II) complexes of synthetic bile acid derivatives. *Bioorg. Med. Chem.* **2006**, *14*, 5204–5210.
(13) Lampronti, I.; Martello, D.; Bianchi, N.; Borgatti, M.; Lambertini, E.; Piva, R.; Jabbar, S.; Choudhuri, M. S. K.; Khan, M. T. H.; Gambari, R. In vitro antiproliferative effects on human tumor cell lines of extracts from the Bangladeshi medicinal plant *Aegle marmelos* Correa. *Phytomedicine* **2003**, *10*, 1300–1308.
(14) Osti, F.; Corradini, F. G.; Hanau, S.; Matteuzzi, M.; Gambari, R. Human leukemia K562 cells: induction to erythroid differentiation by guanine, guanosine and guanine nucleotides. *Haematologica* **1997**, *82*, 395–401.
(15) Olivieri, N. F. Reactivation of fetal hemoglobin in patients with beta-thalassemia. *Semin. Hematol.* **1996**, *33*, 24–42.
(16) Bianchi, N.; Chiarabelli, C.; Borgatti, M.; Mischiati, C.; Fibach, E.; Gambari, R. Accumulation of gamma-globin mRNA and induction of erythroid differentiation after treatment of human leukaemic K562 cells with tallimustine. *Br. J. Haematol.* **2001**, *113*, 951–961.
(17) Bianchi, N.; Onagro, F.; Chiarabelli, C.; Gualandi, L.; Mischiati, C.; Bergamini, P.; Gambari, R. Induction of erythroid differentiation of human K562 cells by cisplatin analogs. *Biochem. Pharmacol.* **2000**, *60*, 31–40.
(18) Bianchi, N.; Osti, F.; Rutigliano, C.; Corradini, F. G.; Borsetti, E.; Tomassetti, M.; Mischiati, C.; Feriotto, G.; Gambari, R. The DNA-binding drugs mithramycin and chromomycin are powerful inducers of erythroid differentiation of human K562 cells. *Br. J. Haematol.* **1999**, *104*, 258–265.
(19) Gambari, R.; Fibach, E. Medicinal chemistry of fetal hemoglobin inducers for treatment of beta-thalassemia. *Curr Med Chem* **2007**, *14*, 199–212.
(20) Chiarabelli, C.; Bianchi, N.; Borgatti, M.; Prus, E.; Fibach, E.; Gambari, R. Induction of gamma-globin gene expression by tallimustine analogs in human erythroid cells. *Haematologica* **2003**, *88*, 826–827.

(21) Fibach, E.; Bianchi, N.; Borgatti, M.; Prus, E.; Gambari, R. Mithramycin induces fetal hemoglobin production in normal and thalassemic human erythroid precursor cells. *Blood* **2003**, *102*, 1276–1281.
(22) Fibach, E.; Bianchi, N.; Borgatti, M.; Zuccato, C.; Finotti, A.; Lampronti, I.; Prus, E.; Mischiati, C.; Gambari, R. Effects of rapamycin on accumulation of alpha-, beta- and gamma-globin mRNAs in erythroid precursor cells from beta-thalassaemia patients. *Eur. J. Haematol.* **2006**, *77*, 437–441.
(23) Zuccato, C.; Bianchi, N.; Borgatti, M.; Lampronti, I.; Massei, F.; Favre, C.; Gambari, R. Everolimus is a potent inducer of erythroid differentiation and gamma-globin gene expression in human erythroid cells. *Acta Haematol.* **2007**, *117*, 168–176.
(24) Lampronti, I.; Bianchi, N.; Borgatti, M.; Fibach, E.; Prus, E.; Gambari, R. Accumulation of gamma-globin mRNA in human erythroid cells treated with angelicin. *Eur. J. Haematol.* **2003**, *71*, 189–195.
(25) Bianchi, N.; Zuccato, C.; Lampronti, I.; Borgatti, M.; Gambari, R. Fetal hemoglobin inducers from the natural world: a novel approach for identification of drugs for the treatment of β-thalassemia and sickle-cell anemia. *Evidence-Based Complementary Alternative Med. (eCAM)*, published online December 11, 2007, doi:10.1093/ecam/nem139.
(26) Menchise, V.; De Simone, G.; Tedeschi, T.; Corradini, R.; Sforza, S.; Marchelli, R.; Capasso, D.; Saviano, M.; Pedone, C. Insights into peptide nucleic acid (PNA) structural features: the crystal structure of a D-lysine-based chiral PNA−DNA duplex. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 12021–12026.
(27) Sforza, S.; Tedeschi, T.; Corradini, R.; Marchelli, R. Induction of helical handedness and DNA binding properties of peptide nucleic acids (PNAs) with two stereogenic centres. *Eur. J. Org. Chem.* **2007**, 5879–5885.
(28) Corradini, R.; Sforza, S.; Tedeschi, T.; Totsingan, F.; Marchelli, R. Peptide nucleic acids with a structurally biased backbone: effect of conformational constraints and stereochemistry. *Curr. Top. Med. Chem.* **2007**, *7*, 681–694.
(29) Betts, L.; Josey, J. A.; Veal, J. M.; Jordan, S. R. A nucleic acid triple helix formed by a peptide nucleic acid−DNA complex. *Science* **1995**, *270*, 1838–1841.
(30) Kokko, J. P.; Mandell, L.; Goldstein, J. H. NMR [nuclear magnetic resonance] investigation of proton mobility in substituted uracils. *J. Am. Chem. Soc.* **1962**, *84*, 1042–1047.
(31) (a) Thompson, M. A. *ArgusLab 4.0*; Planaria Software LLC: Seattle, WA. (b) Thompson, M. A.; Zerner, M. C. A theoretical examination of the electronic structure and spectroscopy of the photosynthetic reaction center from *Rhodopseudomonas viridis*. *J. Am. Chem. Soc.* **1991**, *113*, 8210–8215. (c) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. Development and use of quantum mechanical molecular models. 76. AM1: a new general purpose quantum mechanical molecular model. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.
(32) Theodorou, V.; Ragoussis, V.; Strongilos, A.; Zelepos, E.; Eleftheriou, A.; Dimitriou, M. A convenient method for the preparation of primary amines using tritylamine. *Tetrahedron Lett.* **2005**, *46*, 1357–1360.
(33) Feriotto, G.; Finotti, A.; Breveglieri, G.; Treves, S.; Zorzato, F.; Gambari, R. Transcriptional activity and Sp 1/3 transcription factor binding to the P1 promoter sequences of the human AbetaH-J-J locus. *FEBS J.* **2007**, *274*, 4476–4490.
(34) Lambertini, E.; Piva, R.; Khan, M. T.; Lampronti, I.; Bianchi, N.; Borgatti, M. Effects of extracts from Bangladeshi medicinal plants on in vitro proliferation of human breast cancer cell lines and expression of estrogen receptor alpha gene. *Int. J. Oncol.* **2004**, *24*, 419–423.
(35) Penolazzi, L.; Zennaro, M.; Lambertini, E.; Tavanti, E.; Torreggiani, E.; Gambari, R.; Piva, R. Induction of estrogen receptor alpha expression with decoy oligonucleotide targeted to NFATc1 binding sites in osteoblasts. *Mol. Pharmacol.* **2007**, *71*, 1457–1462.
(36) Borgatti, M.; Bezzerri, V.; Mancini, I.; Nicolis, E.; Dechecchi, M. C.; Lampronti, I.; Rizzotti, P.; Cabrini, G.; Gambari, R. Induction of IL-6 gene expression in a CF bronchial epithelial cell line by *Pseudomonas aeruginosa* is dependent on transcription factors belonging to the Sp1 superfamily. *Biochem. Biophys. Res. Commun.* **2007**, *357*, 977–983.
(37) Borgatti, M.; Bezzerri, V.; Mancini, I.; Nicolis, E.; Dechecchi, M. C.; Rizzotti, P.; Lampronti, I.; Rizzotti, P.; Gambari, R.; Cabrini, G. Silencing of genes coding for transcription factors: biological effects of decoy oligonucleotides on cystic fibrosis bronchial epithelial cells. *Minerva Biotecnol.* **2008**, *20*, 79–84.
(38) Coutouli-Argyropoulou, E.; Zachariadou, C. Synthesis of 5-substituted uracils and 2,4-dimethoxypyrimidines by Wittig olefination. *J. Heterocycl. Chem.* **2005**, *42*, 1135–1142.

JM800982Q