Notes

Retinoic Acid Conjugates as Potential Antitumor Agents: Synthesis and Biological Activity of Conjugates with Ara-A, Ara-C, 3(2*H*)-Furanone, and Aniline Mustard Moieties

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Received March 25, 1996⊗

In a dual targeting approach, to explore the ability of tretinoin (*all-trans*-retinoic acid) to behave as a covalent carrier for cytotoxic entities, conjugates of retinoic acid with a few representative molecules, being important examples of antitumor pharmacophores (i.e., nucleoside analogues and alkylating agents), have been synthesized and tested for their cytostatic and differentiating activity. All compounds were stable to *in vitro* hydrolysis in human plasma and more lipophilic than the parent compounds, thus consenting enhanced uptake into the cells. Among the nucleoside analogues the Ara-C derivatives **3** and **6** and the Ara-A derivative **7** proved the most cytostatic (IC_{50} < 0.32 μg/mL) resulting from 25- to >144-fold more active (Ara-A derivatives) or at least as equally active (Ara-C derivatives) as compared to the parent nucleosides. Compound **3**, endowed with a highly lipophilic silyl moiety at the 3′ and 5′ positions, showed the highest differentiating activity (54% and 44% differentiated HL-60 cells at 0.2 and 0.05 μg/mL respectively). With regard to the retinoic acid conjugates of alkylating agents, compound **10** was the most cytostatic agent (IC_{50} < 0.32 μg/mL) and the most potent differentiating agent (33−34% at 0.32 and 0.08 μg/mL). These structures may also be regarded as analogs of either retinoic acid or the cytotoxic compound.

Introduction

Retinoids are a class of natural and synthetic compounds structurally related to vitamin A. They have been found to be active agents, experimentally as well as clinically, in the prevention and therapy of tumors.[1] Some retinoids have affinity for several binding proteins (i.e., CRABP and CRBP), which were shown to be important in their transport,and modulate cell growth via a family of well-characterized nuclear retinoic acid receptors (RARs, RXRs, and Z) which regulate gene expression.[2,3] This discovery led to the hypothesis that retinoids may exert their differential biological effects, and thus their particular chemopreventive and chemotherapeutic activity, via regulation of gene function by two major pathways: (a) through direct binding of a retinoid receptor complex to retinoid responsive elements (RAREs and RXRs) on DNA[4] or (b) interacting with other regulatory proteins.[5,6] The antitumor activity of tretinoin (*all-trans*-retinoic acid) may be explained to a large extent by an inhibition of cell proliferation and induction of cellular differentiation, and as clearly demonstrated by Bollag et al., an enhanced antitumor effect might be achieved by its combination with low molecular weight inducers and cytokines.[7]

Retinoic acid itself has been reported to inhibit HIV replication. Therefore, it has appeared of interest by different authors to study retinoic acid covalently linked to nucleosides, in order to enhance the uptake of the prodrug by HIV-1-infected cells and to increase its plasma half-life.[8−10]

Retinoic acid prodrugs of AZT and 3′-thia-2′,3′-dideoxycytidine have been synthesized and tested for their antiviral activity by Aggarwal et al. and Camplo et al.,[9,10] and it was found that these retinoic acid derivatives enhanced the uptake of the prodrug in the infected cells, achieving approximately 4-fold higher intracellular concentrations than AZT itself, and increased the plasma half-life of the parent nucleosides. Surprisingly the observed antiviral activity was similar, in both cases, to the parent nucleosides, but the cytotoxicity was increased by 8- and 6-fold, respectively. However, it must be noted that these authors have not investigated eventual concomitant effects related to the differentiating activity of retinoic acid.

A documented approach to enhance the activity of antitumor agents involves the targeting of the alkylating agents (i.e., simple aniline mustards) to DNA by attaching them to DNA-affinity carriers (i.e., DNA-intercalating ligands such as 9-aminoacridine). This approach led to an increased intrinsic drug potency (up to 100-fold), avoiding some of the common mechanisms of cellular resistance to alkylating agents, and to an

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⊗ Abstract published in *Advance ACS Abstracts,* October 1, 1997.

S0022-2623(96)00232-4 CCC: $14.00

alteration of the pattern of formed DNA lesions and their subsequent repair.[11,12] Moreover, in the field of site-directed drug delivery, the dual targeting approach, which implies the use of carrier molecules that have their own intrinsic effect, is a well-recognized concept for drug targeting strategies.[13] Significant examples can be found in the preparation of conjugates of Ara-C and corticosteroids to give compounds with outstanding antitumor activity[14] and in the use of naturally occurring compounds (i.e., sugars, proteins, and steroids) as covalent carriers of cytotoxic entities.[15−17]

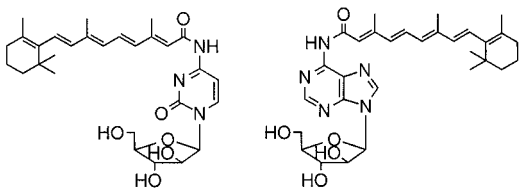
These concepts, together with the well-documented overlapping activities of retinoids, cytokines, and low molecular weight inducers in the regulation of cell growth,[7] have led us to investigate possible conjugates of retinoic acid with antitumor/antiviral agents in the aim to design new potential prodrugs. Moreover, these structures may also be regarded as analogues of either retinoic acid or the cytotoxic compound. In particular, we have selected a few representative molecules as being important examples of antiviral/antitumor/differentiating pharmacophores.

Several observations support the design and synthesis of these conjugates: (i) there is evidence of interesting synergistic antitumor effects by simple combination of antiproliferative agents and retinoic acid;[18−20] (ii) retinoic acid could be released from its amides and esters by cellular enzymes;[9,10] (iii) the retinoic acid prodrug moiety is endowed with differentiating properties as well as with antiproliferative activity;[1] (iv) the increased lipophilicity of a retinoic acid conjugate may improve both the biotransport of the prodrug through cell membranes and the plasma half-life of the conjugated drug.[9,10]

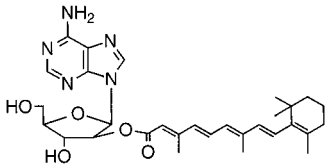
In this study we have evaluated molecular combination of retinoic acid with nucleoside analogues such as Ara-C and Ara-A (**6**−**8**): both latter compounds are potent antitumor and/or antiviral agents; moreover, Ara-C and retinoic acid are also potent differentiating agents,[18−20] used in the experimental therapy of cancer. Ara-C and Ara-A are characterized by good activity but low distribution and high sensitivity to enzymatic degradation in plasma, which can be both improved by masking the amino group with lipophilic functions.[13,14] Moreover, it is known that cellular enzymes hydrolyze nucleoside prodrugs; thus, half-lives of conjugates were evaluated in human plasma. Finally, conjugation of retinoic acid to positions different than 5′ of the nucleoside (N^4-retinoyl-Ara-C, N^6- and 2′-retinoyl-Ara-A) might permit phosphorylation of the conjugates by cellular kinase.[21,22]

With regard to the alkylating moieties (**10**, **12**), the 3(2*H*)-furanone ring is the central common structural feature of a class of naturally occurring antitumor agents (i.e., geiparvarin, jatrophone, and eremantholides A, B, and C),[11] and the *N*,*N*-bis(2-chloroethyl)aniline mustard is commonly used among the clinical antitumor drugs (i.e., melphalan).

Finally, compound **14**, having two retinoyl amides bound through a propylene bridge, may provide us with further information on the role of the amidic bond on prodrug behavior, in terms of activity and/or bioavailability of the retinoyl amides. Moreover, Tsiftsoglou et al.[23,24] have reported on the importance of the bis-amide moiety as a common structural feature of compounds inducing erythroid differentiation.



6 **7**



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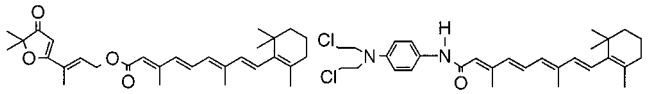
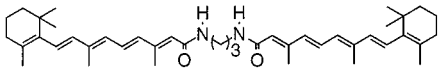


10 **12**



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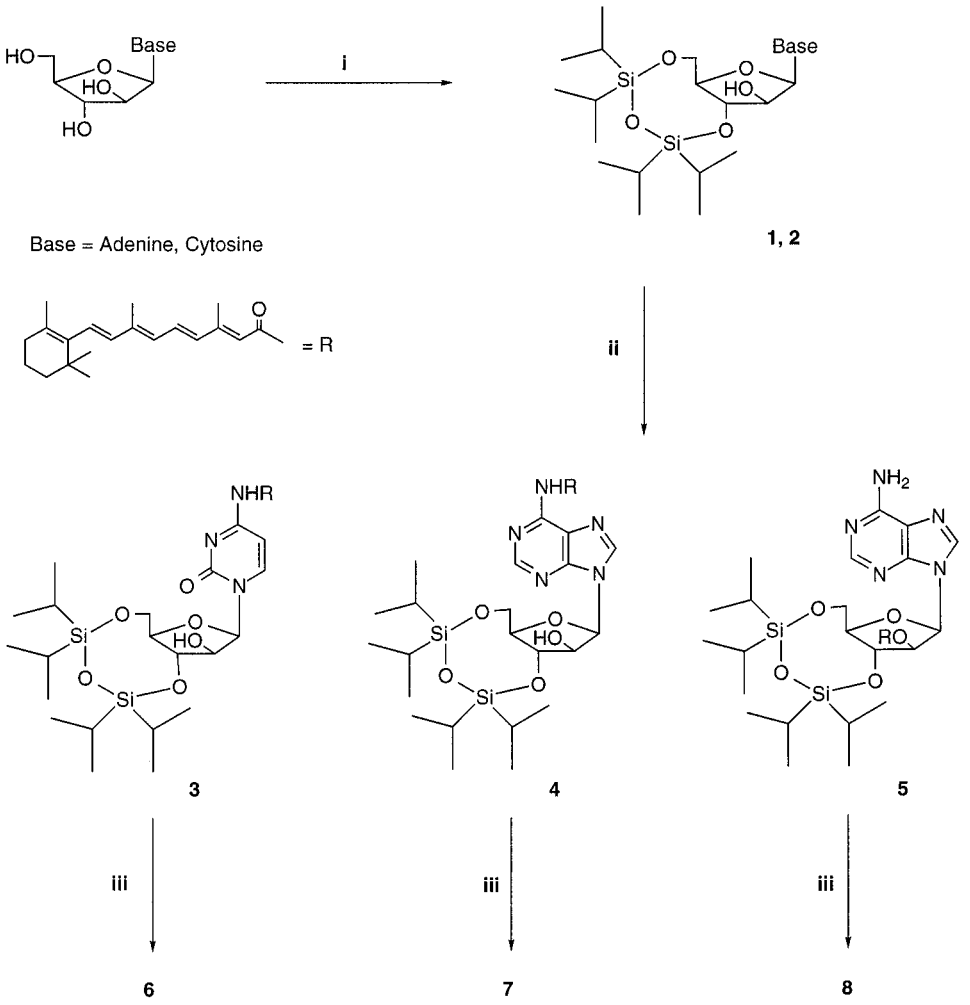
Results

Chemistry. The conjugates **3**−**5**, were obtained by reaction of the corresponding nucleosides (Ara-C and Ara-A), protected as 3′,5′-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl) derivatives (**1** and **2**), and retinoyl chloride *in situ* prepared from retinoic acid and oxalyl chloride in benzene (Scheme 1).[9] The reaction gave both N^6- and 2′-retinoyl derivatives in the case of Ara-A (**4** and **5**) and solely the N^4-retinoyl derivative in the case of Ara-C (**3**). It is interesting to note that removal of the silyl protecting groups, by the use of the standard tetrabutylammonium fluoride (TBAF) procedure, worked well in the case of **4** and **5** but resulted in the loss of the retinoyl moiety in the case of **3**. This drawback was overcome by adopting the ammonium fluoride/methanol[25] (NH_4F/MeOH) procedure in the presence of acetic acid, to give the deprotected **6**−**8** in satisfactory yields (43−95%). Acetic acid was effective, in the case of **3**, as a source of H^+ to prevent the concomitant elimination of the retinoic acid residue at position 4 (due to NH_3 deriving from NH_4F decomposition) and also the possible 2′ → 5′ acyl migration.[21,26] The use of this procedure also avoided residues of TBAF and the difficulty of separating them from the final products.

Structural attributions were based on ^{1}H-NMR, UV spectroscopy, and comparison with other data obtained by us.[22] In particular, in the case of the nucleoside derivatives (**6**−**8**), the regiochemistry of the acylation was attributed on the basis of the ^{1}H-NMR: a ∼1.0 ppm downfield shift for H2′, to ∼5.3 ppm, confirmed the 2′-*O*-acylation.[21] Moreover, the unusual upfield shift of about 0.2 ppm for the C20 methyl, observed in the case of compounds **5** and **8**, might be explained on the basis of a preferred conformation in which the α position of the ester group was in the shielding region of the purine ring. Finally, in the case of Ara-A, the indication that

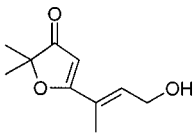
Scheme 1[a]



[a] (i) TPDS-Cl_2, pyridine; (ii) retinoic acid, $C_2O_4Cl_2$, benzene; (iii) NH_4F/MeOH.

the amino group of **8** is free also was derived from the UV spectra [λ_{max}(MeOH) 258 nm].[22] The same considerations could be drawn the case of Ara-C (**6**) [λ_{max}(MeOH) 249 nm].[27,28]

For derivatives **10**, **12**, and **14**, the starting compounds were prepared as described by us (**9**)[29] or by literature procedure (**11**),[30] and diamine **13** was commercially available. Retinoylations were conducted as described above to give the expected compounds in moderate to good yields (45−90%).



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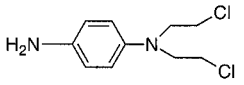

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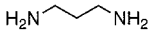

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Determination of *in Vitro* Hydrolysis. To evaluate the susceptibility of the compounds to spontaneous hydrolysis, the prodrugs (**6**−**8**, **10**, **12**, **14**) were incubated in phosphate buffer (pH 7.4), free of plasma enzymes, and in culture medium containing 20% fetal bovine serum for up to 8 h at 37 °C, and all proved fully stable. All compounds were also remarkably stable ($t_{1/2}$ >> 360 min) toward plasma enzymes: upon incubation in human plasma at 37 °C, they did not undergo significant enzymatic hydrolysis for up to 6 h (data not shown).

Biology. Among the retinoic acid conjugates of nucleoside analogues, the Ara-C derivatives **3** and **6** and the Ara-A derivative **7** proved most cytostatic. They inhibited HL-60 cell proliferation at concentrations below 0.32 μg/mL (Table 1), thus being approximately 144-fold more cytostatic as (compound **7**) or comparably active to (compounds **3** and **6**) the reference compounds Ara-A and Ara-C, respectively. In contrast, the Ara-A derivatives **4** and **8** proved markedly less active but at least 25-fold more cytostatic than the parent Ara-A. The compounds were also evaluated on their differentiating potential against HL-60 cell cultures. A concentration close to their IC_{50} value and a 4-fold lower concentration have been chosen to examine the effect of the test compounds on differentiation. The Ara-C derivative **3**, in which retinoic acid was conjugated at the amino group of the base moiety and in which the 3′ and 5′ positions of the sugar moiety were linked by highly lipophilic silyl moieties, proved superior to the Ara-C derivative **6**, in which the 3′- and 5′-hydroxyl moieties were not substituted. Indeed, 0.2 and 0.05 μg/mL **3** resulted in 54% and 44% differentiated HL-60 cells, respectively, whereas 0.32 and 0.08 μg/mL **6** resulted in 37% and 29% differentiated cells. However, the Ara-A derivative **7** was almost as potent as **3**, as a differentiating agent, whereas its silylated derivative was much less active in differentiating the HL-60 cell cultures. Also, compound **8** in which retinoic acid was

Table 1. Cytostatic and Differentiating Activity of Retinoic Acid Conjugates against HL-60 Cells

		differentiation	
compound	cytostatic activity IC_{50}, μg/mL (μM)[a]	concentration, μg/mL (μM)	differentiation (% NBT-positive cells)
3	<0.32 (0.41)	0.2 (0.26)	54
		0.05 (0.065)	44
4	2.08 (2.85)	2 (2.52)	31
		0.5 (0.63)	31
6	<0.32 (0.6)	0.32 (0.6)	37
		0.08 (0.15)	29
7	<0.32 (0.54)	0.2 (0.32)	46
		0.05 (0.08)	41
8	1.96 (3.30)	2 (3.36)	36
		0.5 (0.84)	34
10	<0.32 (0.68)	0.32 (0.68)	34
		0.08 (0.17)	33
12	3.7 (7.19)	4 (7.76)	43
		1 (1.94	24
14	3.71 (5.53)	4 (5.96)	65
		1 (1.49)	33
ara-C	0.029 (0.12)	0.4 (1.65)	20
		0.08 (0.33)	5
		0.016 (0.066)	8
ara-A	20.9 (78)	50 (187.1)	12
		10 (37.4)	9
DMSO		5.6 (71.8, 0.5%)	53
		2.8 (35.9, 0.25%)	28
		1.4 (17.9,0.125%)	12
retinoic acid		0.03 (0.1)	89
		0.003 (0.01)	67
		0.0003 (0.001)	34
control			12

[a] 50% inhibitory concentration. The cytotoxicity of compounds **9** and **11** has already been reported (refs 29 and 11), and they do not express any differentiating activity.

conjugated at the 2′-hydroxyl of Ara-A proved to be a poorer differentiating agent. In all cases the differentiation potential was increased with regard to the free nucleosides, but it should be mentioned that retinoic acid itself was at least 2 to 3 orders of magnitude superior to the conjugates to differentiate the HL-60 cell culture.

With regard to the retinoic acid conjugates of alkylating agents, compound **10** proved most cytostatic (IC_{50} < 0.32 μg/mL) and also had the strongest differentiating potential (33−34% at 0.32 and 0.08 μg/mL). Among compounds **12** and **14**, which were equally cytostatic, compound **14**, which contained 2 units of retinoic acid, proved slightly superior to **12** with regard to its differentiating potential (65% and 33% at 4 and 1 μg/mL, respectively).

Discussion

The objective of this study was to design and synthesize molecular combinations of retinoic acid and cytotoxic entities. Synergistic or additive effects might be expected from the release of the two components of the prodrugs. Indeed, the elevated half-life value ($t_{1/2}$ >> 360 min) observed accounts for a good distribution of the prodrugs and thus for possible candidates for *in vivo* testing. However, only in the case of Ara-A derivatives (compounds **4**, **7**, and **8**) could a 25- to >144-fold increment in the cytostatic activity could be observed.

From our experimental data, there is no clear-cut correlation between cytostatic activity and differentiating potential of the test compounds. Also, there seems to be no predictable structure−activity relationship (SAR) for the differentiating potential of the test compounds. The most striking examples are the differences in differentiating activity of the Ara-C and Ara-A derivatives containing or lacking the lipophilic silyl groups in the sugar moiety. For the Ara-C derivatives **3** and **6**, an inverse correlation was found compared with the Ara-A derivatives **4** and **7**. Several factors may contribute to this lack of SAR. Conjugation of the retinoic acid moiety to different parts of the Ara-C or Ara-A molecule may result in differential loss of the differentiating properties of both retinoic acid and/or the nucleosides. Since Ara-C and Ara-A presumably have to be phosphorylated before they can act as differentiating agents on their own right, it will depend on the intracellular stability of the test compounds as well as their recognition by nucleoside kinases to what extent the eventual differentiating properties will be expressed. Also, the interaction of the retinoic acid receptor in HL-60 cells with retinoic acid, required for optimal differentiation, will differ from one conjugate to another and will severely affect the differentiating potential of retinoic acid conjugates.

Among the alkylating entities, the activity of compound **10** is particularly interesting. Indeed the linkage of the furanone **9** to retinoic acid resulted in the most pronounced activity among the derivatives of this simple 3(2*H*)-furanone alkylating moiety[29] which is structurally related to the antitumor agent geiparvarin. Moreover, it is particularly surprising to note the lack of activity of the mustard derivative **12**, since this chemical moiety usually results in an *in vitro* cytotoxic and *in vivo* antitumor effect. Our data that free retinoic acid is a much more potent differentiating agent than the retinoic acid conjugates are in agreement with a lower efficiency of interaction of conjugated retinoic acid with its receptor. However, in the case of Ara-A derivatives significant (25- to >144-fold) increments in the cytostatic effects were observed, but this was not the case for Ara-C derivatives. These data are partially in contrast with the reported, overall increased cytotoxicity (6−8-fold) of AZT and 3′-thia-2′,3′-dideoxycytidine retinoic acid conjugates which require intracellular hydrolysis prior to expression of their biological activity,[9,10] but these authors did not report the possible effects on the differentiating activity as compared to retinoic acid itself.

In conclusion, although no clear SAR emerged from this study, the known greater instability of the *N*-nucleoside conjugates of Ara-C and Ara-A, resulting in a partial release inside the cells of retinoic acid and the nucleoside analogue, may be at the basis of their observed higher activity in comparison with the other conjugates, especially in the case of Ara-A. However, it must be noted that the prodrug potential of our conjugates depends on how efficiently they can be concentrated inside the target cells, due to both the increased plasma stability and the transport across the cell membrane, and then reversed to the parent compounds. Indeed, Ara-A conjugates showed an interesting 25- to >144-fold increased cytostatic activity, as compared to Ara-A, but with reduced differentiating activity, as compared to retinoic acid. This occurrence remains so far unexplained but may be also consistent with a possible cytostatic activity expressed by the intact conjugates.

The low activity of the mustard **12** and the diamide **14** indicates that these molecules may act, as intact entities, as retinoic acid analogues rather than as carriers for the two moieties, this being partially in contrast with the reported prominent retinoidal activity of simple as well as complex retinamides.[31,32]

Experimental Section

Chemistry. Material and Methods. The reaction course and product mixture were routinely monitored by thin-layer chromatography (TLC) on silica gel-precoated F254 Merck plates with detection under 254-nm UV lamp and/or by spraying the plates with 10% H_2SO_4/MeOH and heating. After purification (column chromatography was performed with ICN 60−200 mesh silica gel), all compounds gave analytical data consistent with the expected structures. ^{1}H-NMR spectra were determined at 200 MHz for compound solutions in $CDCl_3$ or DMSO-d_6 with a Brucker AC-200 spectrometer. Ultraviolet spectra were recorded on a Kontron UVIKON 922 spectrometer. HPLC analyses were conducted on a Waters 600E chromatographic system, using reverse-phase Waters C18 columns (150 × 4.6 mm, 150 Å). Elemental analyses, unless otherwise noted, were within ±0.4% of the theoretical values.

Preparation of the Protected Ara-C and Ara-A (1, 2). 3′,5′-*O*-TPS-Ara-C and -Ara-A were obtained by standard procedure. **1:** syrup; yield 63%.[33] **2:** syrup; yield 67%.[34]

General Procedure for the Preparation of Retinoic Acid Conjugates (3−5, 10, 12, 14). To a solution of retinoic acid (0.106 g, 0.36 mmol) in dry benzene (10 mL) was added oxalyl chloride (46 mL, 0.54 mmol), and the reaction mixture was stirred, at ambient temperature and protected from light, for 2 h under positive argon pressure. The deep-yellow solution was then evaporated under high vacuum (1×10^{-3} bar), and the residue was redissolved in dry benzene (5 mL) and slowly added, at 0 °C and under argon positive pressure, to a solution of the appropriate compound **3**−**5**, **9**, **11** (0.36 mmol), and **13** (0.18 mmol), in dry benzene containing DMAP (catalytic amount) and TEA (1.5 mL, 1.8 mmol). After 1 h (TLC analysis) the reaction was usually complete. The reaction mixture was then diluted with benzene (20 mL) and evaporated to dryness to give a residual oil which was purified by silica gel column chromatography.

3: eluent CH_2Cl_2/MeOH, 9.5/0.5; yellow foam; yield 40%; ^{1}H-NMR (DMSO-d_6) δ 0.92−1.08 (m, 34H, iPr × 4, CH_3 × 2 cyclohexenyl), 1.41−1.50 (m, 2H, CH_2 cyclohexenyl), 1.55−1.63 (m, 2H, CH_2 cyclohexenyl), 1.72 (s, 3H, CH_3), 1.90−2.10 (m, 5H, CH_2 cyclohexenyl and CH_3); 2.36 (s, 3H, CH_3); 3.78−4.20 (m, 4H, H3′,4′,5′,5″), 4.30−4.50 (m, 1H, H2′), 5.61 (pseudo-t, 1H, OH2′), 5.70 (d, J = 7.5 Hz, 1H, H-5), 5.74 (br s, 2H, H1′, retinoyl), 6.12−6.43 (m, 4H, retinoyl), 6.95−7.18 (m, 2H, NH, retinoyl), 7.50 (d, 1H, H-6), 8.45 (br s, 1H, NH).

4: eluent EtOAc/hexane, 3/7 → 4/7; yellow foam; yield 45%; ^{1}H-NMR ($CDCl_3$) δ 0.95−1.10 (m, 34H, iPr × 4, CH_3 × 2 cyclohexenyl), 1.45−1.50 (m, 2H, CH_2 cyclohexenyl), 1.61−1.69 (m, 2H, CH_2 cyclohexenyl), 1.72 (s, 3H, CH_3), 2.0−2.10 (m, 5H, CH_2 cyclohexenyl, CH_3), 2.36 (s, 3H, CH_3), 3.60−3.65 (m, 1H, H5′), 4.0−4.10 (m, 2H, H4′, H5″), 4.32−4.39 (dd, 1H, J = 8.5, 3 Hz, H3′), 4.55−4.60 (m, 1H, H2′), 5.55 (br s, 1H, OH2′), 5.94 (d, 1H, J = 5 Hz, H1′), 6.09−6.37 (m, 6H, retinoyl, NH), 6.50 (s, 1H, H2), 7.0−7.14 (m, 1H, retinoyl), 8.14 (s, 1H, H8).

5: eluent EtOAc/hexane, 3/7 → 4/7; yellow foam; yield 25%; ^{1}H-NMR ($CDCl_3$) δ 1.01−1.25 (m, 34H, iPr × 4, CH_3 × 2 cyclohexenyl), 1.40−1.50 (m, 2H, CH_2 cyclohexenyl), 1.55−1.65 (m, 2H, CH_2 cyclohexenyl), 1.70 (s, 3H, CH_3), 1.90−2.05 (m, 5H, CH_2 cyclohexenyl, CH_3), 2.19 (s, 3H, CH_3), 3.92−4.0 (m, 1H, H4′), 4.10−4.20 (m, 1H, H5′), 4.20−4.35 (m, 1H, H5″), 4.95−5.10 (m, 1H, H3′), 5.50−5.60 (m, 1H, H2′), 5.70 (br s, 2H, NH_2), 6.14−6.35 (m, 5H, retinoyl), 6.49 (d, 1H, J = 5.5 Hz, H1′), 6.85−7.0 (m, 1H, retinoyl), 7.98 (s, 1H, H2), 8.28 (s, 1H, H8).

10: eluent EtOAc/hexane, 1/9 → 2/8; oil; yield 45%; UV (MeOH) λ_{max} 360 (ϵ 20 000), 297 (ϵ 17 000), λ_{min} 325 (ϵ 15 000), 263 (ϵ 11 000); ^{1}H-NMR ($CDCl_3$) δ 1.03 (s, 6H, CH_3 × 2 cyclohexenyl), 1.40 (s, 6H, CH_3 × 2 furan), 1.41−1.49 (m, 2H, CH_2 cyclohexenyl), 1.55−1.65 (m, 2H, CH_2 cyclohexenyl), 1.71 (s, 3H, CH_3 cyclohexenyl), 1.98−2.09 (m, 8H, CH_2 cyclohexenyl, CH_3 retinoyl, CH_3 alkenyl side chain), 2.38 (s, 3H, CH_3), 4.85 (d, J = 6.3 Hz, 2H, CH_2O), 5.58 (s, 1H, furanyl), 5.82 (s, 1H, retinoyl), 6.10−6.33 (m, 4H, retinoyl), 6.60−6.70 (m, 1H, alkenyl side chain), 6.95−7.08 (m, 1H, retinoyl). Anal. ($C_{30}H_{40}O_4$) C, H.

12: eluent EtOAc/hexane 4/6 → 1/1; brown gum; yield 90%; UV (MeOH) λ_{max} 348 (ϵ 45 000), 262 (ϵ 15 000), λ_{min} 285 (ϵ 11 000), 227 (ϵ 10 000); ^{1}H-NMR ($CDCl_3$) δ 1.03 (s, 6H, CH_3 × 2 cyclohexenyl), 1.40−1.50 (m, 2H, CH_2 cyclohexenyl), 1.55−1.65 (m, 2H, CH_2 cyclohexenyl), 1.71 (s, 3H, CH_3 cyclohexenyl), 1.90−2.10 (m, 5H, CH_2 cyclohexenyl, CH_3), 2.39 (s, 3H, CH_3), 3.53−3.62 (m, 8H, CH_2 × 4), 5.82 (s, 1H, retinoyl), 6.05−6.30 (m, 4H, retinoyl), 6.55 (d, 2H, J = 8 Hz, aryl), 6.85−7.00 (m, 1H, retinoyl), 7.43 (d, 2H, aryl), 8.08 (s, 1H, NH). Anal. ($C_{30}H_{39}Cl_2N_2O$) C, H, N.

14: eluent EtOAc/hexane, 1/1; yellow solid, mp 89 °C; yield 67%; UV (MeOH) λ_{max} 349 (ϵ 31 700), λ_{min} 252 (ϵ 5000); ^{1}H-NMR ($CDCl_3$) δ 1.02 (s, 12H, CH_3 × 4 cyclohexenyl), 1.40−1.50 (m, 4H, CH_2 × 2 cyclohexenyl), 1.55−1.67 (m, 6H, CH_2 × 2 cyclohexenyl, CH_2 diaminopropyl), 1.71 (s, 6H, CH_3 × 2 cyclohexenyl), 1.90−2.10 (m, 10H, CH_2 × 2 cyclohexenyl, CH_3 × 2), 2.36 (s, 6H, CH_3 × 2), 3.30−3.42 (m, 4H, N−CH_2 × 2 diaminopropyl), 5.76 (s, 2H, retinoyl × 2), 6.07−6.28 (m, 8H, retinoyl × 2), 6.80−6.91 (m, 4H, retinoyl × 2, NH × 2). Anal. ($C_{43}H_{62}N_2O_2$) C, H, N.

General Procedure for Deprotection of the Nucleoside Derivatives 3−5. To a solution of the protected **3**−**5** (0.26 mmol) in dry MeOH (10 mL) under positive argon pressure was added acetic acid (0.039 g, 0.65 mmol) followed by NH_4F (0.115 mg, 3 mmol). The reaction was monitored on TLC (CH_2Cl_2/MeOH, 9/1) and heated at reflux conditions if necessary. After evaporation to dryness the residue was purified by silica gel column chromatography.

6: eluent CH_2Cl_2/MeOH, 9.5/0.5; orange solid, mp 111 °C; yield 43%; UV (MeOH) λ_{max} 370 (ϵ 32 000), 249 (ϵ 14 000), 298 (ϵ 8000), λ_{min} 270 (ϵ 4000), 230 (ϵ 6000); ^{1}H-NMR (DMSO-d_6) δ 1.02 (s, 6H, CH_3 × 2 cyclohexenyl), 1.40−1.50 (m, 2H, CH_2 cyclohexenyl), 1.54−1.65 (m, 2H, CH_2 cyclohexenyl), 1.71 (s, 3H, CH_3), 1.95−2.05 (m, 5H, CH_2 cyclohexenyl, CH_3), 2.34 (s, 3H, CH_3), 3.45−3.60 (m, 2H, H5′,5″), 3.65−3.75 (m, 1H, H4′), 3.80−4.0 (m, 2H, H3′,2′), 5.05 (br s, 1H, OH5′), 5.40−5.45 (m, 2H, OH3′,2′), 6.05−6.40 (m, 6H, H1′, retinoyl), 6.90−7.03 (m, 1H, retinoyl), 7.20 (d, 1H, J = 7.5 Hz, H-5), 8.05 (d, 1H, H-6), 10.45 (br s, 1H, NH). Anal. ($C_{29}H_{40}N_3O_6$) C, H, N.

7: eluent CH_2Cl_2/MeOH, 9/1; syrup; yield 66%; UV (MeOH) λ_{max} 372 (ϵ 29 000), 216 (ϵ 21 500), λ_{min} 294 (ϵ 7700), $\lambda_{shoulder}$ 240 (ϵ 10 000); ^{1}H-NMR (DMSO-d_6) δ 1.03 (s, 6H, CH_3 × 2 cyclohexenyl), 1.40−1.50 (m, 2H, CH_2 cyclohexenyl), 1.54−1.62 (m, 2H, CH_2 cyclohexenyl), 1.69 (s, 3H, CH_3), 1.95−2.02 (m, 5H, CH_2 cyclohexenyl, CH_3), 2.33 (s, 3H, CH_3), 3.60−3.69 (m, 2H, H5′,5″), 3.74−3.85 (m, 1H, H4′), 4.10−4.28 (m, 2H, H3′,2′), 5.10−5.18 (br, 1H, OH5′), 5.70−5.75 (br, 2H, OH2′,3′), 6.01−6.43 (m, 6H, H1′, retinoyl × 5), 6.75 (s, 1H, H2), 6.96−7.10 (m, 1H, retinoyl), 8.09 (s, 1H, H8), 10.05 (br s, 1H, NH). Anal. ($C_{30}H_{39}N_5O_5$) C, H, N.

8: eluent CH_2Cl_2/MeOH, 9.5/0.5; syrup; yield 95%; UV (MeOH) λ_{max} 366 (ϵ 27 000), 258 (ϵ 15 500), λ_{min} 290 (ϵ 8000), 229 (ϵ 8500); ^{1}H-NMR (DMSO-d_6) δ 1.03 (s, 6H, CH_3 × 2 cyclohexenyl), 1.40−1.50 (m, 2H, CH_2 cyclohexenyl), 1.54−1.62 (m, 2H, CH_2 cyclohexenyl), 1.72 (s, 3H, CH_3), 1.85−2.05 (m, 5H, CH_2 cyclohexenyl, CH_3), 2.17 (s, 3H, CH_3), 3.60−3.80 (m, 2H, H5′,5″), 3.83−3.88 (m, 1H, H4′), 4.48 (q, 1H, J = 5.8, 5.9 Hz, H3′), 5.15 (br, 1H, OH5′), 5.30 (ϕt, 1H, J = 5.8, 5.7 Hz, H2′), 5.54 (s, 1H, retinoyl), 5.86 (d, 1H, J = 5.2 Hz, OH3′), 6.09−6.30 (m, 4H, retinoyl), 6.46 (d, 1H, J = 5.7 Hz, H1′), 6.93−7.01 (m, 1H, retinoyl), 7.26 (br s, 2H, NH_2), 8.09 (s, 1H, H2), 8.20 (s, 1H, H8). Anal. ($C_{30}H_{39}N_5O_5$) C, H, N.

Hydrolysis of the Compounds. To 100 μL of phosphate buffer (0.2 M, pH 7.4), human plasma, or culture medium containing 20% fetal bovine serum was added 10 μL of a solution of one of the compounds (10 mg/mL in DMSO), and the mixture was incubated at 37 °C in a water bath. At various time intervals (0−8 h), 20 μL of the samples was withdrawn and introduced in a quartz cuvette containing 1

mL of water. Optical density values were measured at the following wavelengths: **6** at 362, 351, and 274 nm; **7** at 376, 351, and 251 nm; **8** at 366, 351, and 259 nm; **10** at 360, 351 and 261 nm. In the case of **12** and **14** (having λ_{max} overlapping with that of the retinoic acid) the hydrolyses were monitored by HPLC: 20 μL of samples was withdrawn, diluted with methanol (800 μL), and poured at −21 °C. The supernatants were filtered and analyzed by HPLC using an acetonitrile−water solvent system.

Biology. Inhibition of Tumor Cell Growth. HL-60 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). All assays were performed in 96-well microtiter plates. To each 200-μL well were added 7.5×10^4 HL-60 cells (100 μL) and a given amount of the test compound (100 μL). The cells were then allowed to proliferate for 72 h at 37 °C in a humidified CO_2-controlled atmosphere. The growth of the cells was linear during this incubation period. At the end of the incubation period, the cells were counted in a Coulter counter (Coulter Electronics Ltd., Harpenden, Herts, England). The IC_{50} (50% inhibitory concentration) was defined as the concentration of compound that reduced the number of tumor cells by 50%.

Differentiation of HL-60 Cell Cultures. The differentiating activity of the test compounds was examined in HL-60 cell cultures and performed as previously described.[35] Briefly, 106 HL-60 cells (exposed to the test compounds for 6 days) were suspended in 1 mL of RPMI-1640 culture medium containing 20% fetal bovine serum and 2 mM L-glutamine. Then 1 mL of a freshly prepared nitro blue tetrazolium (NBT) solution [2 mg/mL, containing phorbol 12-myristate 13-acetate (PMA) at 200 ng/mL in phosphate-buffered saline, pH 7.2] was added to the cell suspension, and the mixture was further incubated for 40 min in a shaking water bath at 37 °C. After this incubation period, the mixture was cooled on ice during 5 min to stop the reaction. Then the cells were centrifuged for 10 min at 1000 rpm at 4 °C, and the supernatant was carefully removed. The cell pellet was resuspended in 0.5 mL of RPMI-1640 medium, kept on ice, and protected from light until the percentage of cells containing dark blue formazan precipitates was determined in a hemocytometer by light microscopy. For each sample, at least a total of 200 cells were scored, and the percentage of formazan-positive cells was calculated. Cell cultures that were not exposed to the test compounds were included in this assay procedure and found to contain not more than 12% NBT-positive cells.

Acknowledgment. This work was supported by grants from the Belgian Nationaal Fonds voor Geneeskundig Wetenschappelijk Onderzoek (3.3010.91), the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (3.0180.95), the Belgian Econcerteerde Onderzoeksacties (95/5), and the Consiglio Nazionale delle Ricerche (Progetto Finalizzato Chimica Fine 2, 93.02895.PS72 and 96.01104). We thank Lizette van Berckelaer for excellent technical assistance.

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JM9602322