Brief Articles

Novel Mutual Prodrug of Retinoic and Butyric Acids with Enhanced Anticancer Activity

Abraham Nudelman*,§ and Ada Rephaeli*,‡

Chemistry Department, Bar Ilan University, Ramat Gan 52900, Israel, and Felsenstein Medical Research Center, Petach Tikva-Sackler School of Medicine, Tel Aviv University, Israel

Received October 28, 1999

Acyloxylalkyl esters of retinoic acid and small carboxylic acids (C3−5) were evaluated for anticancer activity. The derivative of butyric acid (BA) and *all-trans*-retinoic acid (ATRA) − retinoyloxymethyl butyrate (**RN1**) − acting as a mutual prodrug was a more potent inducer of cancer cell differentiation and inhibitor of proliferation than the parent acids. ED_{50} of **RN1** for differentiation induction in HL-60 was over 40-fold lower than that of ATRA. The differentiating activity of ATRA compared to that of the acyloxylalkyl esters derived from butyric (**RN1**), propionic (**RN2**), isobutyric (**RN3**), and pivalic (**RN4**) acids was found to be: **RN1** > **RN2** > **RN3** > ATRA ∼ **RN4**. This observation implies that the activity of the prodrugs depends on the specific acyl fragment attached to the retinoyl moiety, and the butyroyl fragment conferred the highest potency. The IC_{50} values for inhibition of Lewis lung (3LLD122) and pancreatic ($PaCa_2$) carcinoma cell line colony formation elicited by **RN1** were significantly higher than those of ATRA. In addition to its superiority over ATRA or BA as growth inhibitors of the above cell lines, **RN1** was also able to overcome the resistance to ATRA in 3LLD122 cells.

Introduction

Differentiation induction of malignant cells is defined by the ability of an agent to induce a more normal or benign phenotype in these cells. Butyric acid (BA) and *all-trans*-retinoic acid (ATRA) are among the better-known differentiation-inducing agents and offer a potential alternative to conventional cytotoxic cancer treatment.[1−5] ATRA belongs to the family of retinoids that includes active metabolites of vitamin A as well as a diverse spectrum of synthetic derivatives. The endogenous retinoids play a pivotal role in normal development of endo-, meso-, and ecto-dermally derived tissues. Retinoids bind to a cytoplasmic binding protein, whose role in mediating the signals is unclear. In the nucleus, they bind to the RAR and RXR receptors, and the ligand−receptor complex interaction with the retinoid-responsive DNA sequence leads to the activation of target genes' transcription.[6−8] Retinoids have been reported to induce differentiation and arrest proliferation in a wide spectrum of cancer cells: e.g. the human myeliod leukemia cell line HL-60 in which expression of cellular and molecular characteristics of granulocytes was induced.[1] Retinoids are currently used for treatment of promyelocytic leukemia and are in clinical studies as potential anticancer agents against other cancer types such as neuroblastoma.[9]

BA is present in the digestive system in millimolar concentrations as a byproduct of bacterial fermentation.[10] It has shown antineoplastic activity in a wide spectrum of cells in vitro; however, in vivo it displays low potency due to rapid metabolism.[11] Studies of primary myeloid leukemic cells have shown that BA is a more effective inhibitor of cell proliferation and inducer of cytodifferentiation than RA, Ara-C, or 1-α,-25-dihydroxy-vitamin D_3.[12] BA is known to specifically inhibit histone deacetylase (HDAC), and many of its biological effects could be attributed to this activity.[4,13,14] The inhibition of HDAC leads to histones hyperacetylation and relaxation of the chromatin structure. The chromatin conformational change allows the access of transcription factors and upregulation of gene expression.[15,16] Recent studies have established a link between oncogene-mediated suppression of transcription and recruitment of HDAC into a nuclear complex.[17−20] BA, 4-phenylbutyric acid, and trichostatin A reverse this suppression by specific inhibition of HDAC activity, leading to histone hyperacetylation, chromatin relaxation, and enhanced transcription.[21−24] Several laboratories have reported that the translocation-generated fusion oncogenes (PML-RAR and PLZF-RAR) in acute promyelocytic leukemia (APL) suppress transcription as a result of sequestering HDAC.[25,26] Resistance to ATRA of human APL cell lines could be overcome by addition of HDAC inhibitors.[24−26] Of particular importance is the observation that an APL patient, who failed multiple therapies and was highly resistant to ATRA, responded to the combination treatment of ATRA and phenylbutyric acid.[24] Synergy between BA and ATRA in an APL cell line was observed.[27]

Previously, we have reported on a family of BA prodrugs, where pivaloyloxymethyl butyrate (**AN9**) is the representative best-studied.[28,29] **AN9** induces tran-

* To whom correspondence should be addressed. For A.N.: Phone: 972-3-531-8314. Fax: 972-3-535-1250. E-mail: nudelman@mail.biu.ac.il.
§ Bar Ilan University.
‡ Tel Aviv University.

10.1021/jm990540a CCC: $19.00
Published on Web 07/08/2000

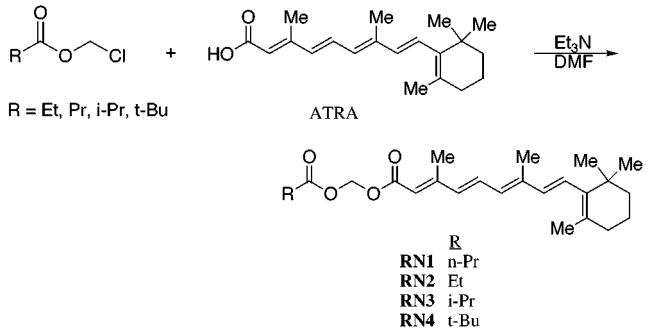
sient hyperacetylation of histones; this activity is likely to be an important mechanism by which these prodrugs affect gene modulation.[30,31] **AN9** was shown to act upon cells at about 10-fold lower concentration and at least 100-fold faster rate than does BA. Moreover, it induces apoptosis in cancer cells in vitro and exhibits in vivo anticancer activity.[29−32] **AN9** belongs to a well-established family of acyloxyalkyl ester prodrugs of carboxylic acids whose expected intracellular hydrolytic degradation products are BA, pivalic acid, and formaldehyde.[33] Whereas pivalic acid is known not to contribute to the activity elicited by the prodrug, the role of the released formaldehyde remains unclear. The pivaloyloxymethyl derivatives of propionic, valeric, and pivalic acids, analogues of **AN9** that lack a BA fragment, were found to have little or no antitumor activity on cancer cells.[29] This suggests that the biological activity of **AN9** stems from the released BA moiety. **AN9**, formulated in lipid emulsion, labeled PIVANEX, displayed low toxicity and was reported to have an estimated MTD of 2.69 g/m^2/day in a phase I clinical study, with advanced solid tumor patients.[34] It is presently in phase II clinical studies with non-small-cell lung carcinoma.

The present study describes the synthesis and activities of a novel mutual prodrug of BA and ATRA which possesses enhanced differentiating and anticancer activities compared to the parent compounds or their combination.

Chemistry

Compounds **RN1**−**RN4** were prepared form ATRA and the chloromethyl esters of the C3−5 aliphatic acids (Scheme 1). At −20 °C the neat compounds were stable

Scheme 1



in the dark for at least 1 month. In AcOEt, EtOH, and DMSO solutions at room temperature, they exhibited instability. Prior to biological evaluation, the compounds were purified by preparative TLC, and the samples used were estimated to be at least 95% pure.

Biological Results

The activity of **RN1**, the mutual prodrug of ATRA and BA, was tested in vitro against three cancer cell lines. Differentiation induction activity was determined in the human myeloid leukemic cell line HL-60, which provides a useful model system for studying differentiation of leukemic cells.[1] HL-60 cells were grown for 2−4 days in the absence or presence of tested differentiating agents. Cell differentiation evaluated by NBT reduction activity correlated well with the appearance of mature cell functions and phenotype. The concentration that induced one-half of the maximum NBT reduction activity (ED_{50}) after 4 days of treatment was determined by linear regression, and the average values from independent experiments were determined (Table 1). The ED_{50} of **RN1** for differentiation induction in HL-60 was 0.031 μM, 40-fold lower than that of ATRA and over 9000-fold lower than BA. In an unpaired *t*-test, the activity of **RN1** was shown to be significantly greater than that of the parent drugs ($p \ll 0.05$).

Table 1. Effect of BA, ATRA, and Their Mutual Prodrug, **RN1**, on Differentiation of HL-60 Cells

compd	differentiation induction, ED_{50} (μM)[a]
BA	285 ± 114
ATRA	1.25 ± 0.05
RN1	0.031 ± 0.008

[a] The ED_{50} for NBT reduction activity after 4 days of treatment was determined by linear regression, and the average values from three independent experiments were measured.

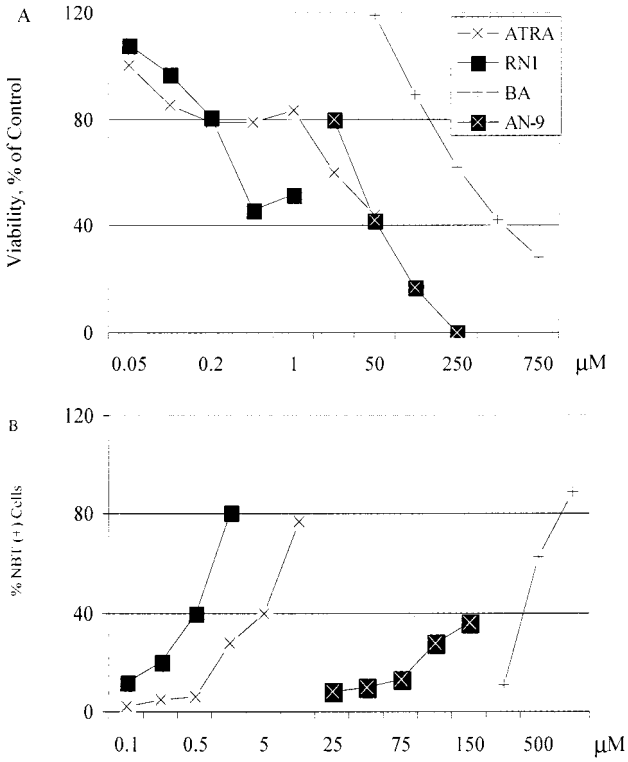


Figure 1. Effect of the compounds on viability (A) and 2-day differentiation (B) of the myeloid leukemic cell line HL-60.

The increased cytodifferentiation was associated with diminished proliferation. Figure 1 shows a representative experiment examining the effect of BA, ATRA, **RN1,** and **AN9** on the viability (A) and differentiation induction (B) of HL-60 cells after 2 days of treatment. In both cases, the same order of potency, **RN1** > ATRA > **AN9** ≫ BA, was observed. Furthermore, the differentiation activity elicited by **RN1** was greater than that of the combined parent acids (Figure 2). Treatment with a mixture of 0.5 μM ATRA and 50 μM BA resulted in more than additive differentiation activity, yet the effect was significantly lower than that obtained with 0.5 μM **RN1**. It might be unexpected that the coupling of BA to ATRA would cause such a large increase in activity, considering the low potency of BA. The results may be explained by a combination of two factors: (1) the ATRA fragment of the **RN1** imparts lipophilicity and facilitates the penetration of BA to the cellular

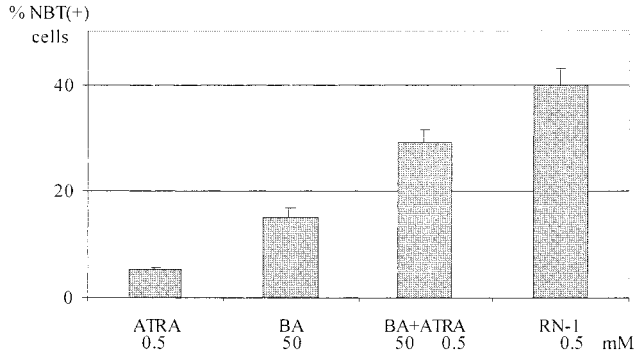


Figure 2. Effect of the compounds on 2-day differentiation of HL-60 cells.

Table 2. SAR Study of the Effect of the Carboxylic Acid Fragment of the Retinoyl Prodrug on Differentiation Activity[a]

expt	**RN1**	**RN2**	**RN3**	ATRA	**RN4**
A	72	60	48	21	13
B	74	56	54	30	21
C	78	57	49	22	17
average	74.6 ± 2.2	57.6 ± 1.5	50.3 ± 2.4	24.3 ± 3.8	17 ± 2.7

[a] HL-60 cells were grown for 2 days in the absence or presence of ATRA**, RN1, RN2, RN3,** and **RN4** (0.25 μM). Differentiation was evaluated by NBT reduction activity in three independent experiments, and values are expressed as % of NBT positive cells, as described in the Experimental Section.

Table 3. Effect of BA, **AN9**, ATRA, and **RN1** on Colony Formation Activity of Lung Carcinoma (3LLD122) and Pancreatic (PACA) Human Cell Lines

	colony formation inhibition, IC_{50} (μM)	
compd	3LLD122[a]	PACA[b]
BA	>1000, $n = 4$	250 ± 169, $n = 3$
AN9	35 ± 6, $n = 3$	30 ± 9, $n = 3$
ATRA	33 ± 10, $n = 3$	9.5 ± 2.4, $n = 3$
RN1	4.5 ± 1, $n = 3$	4.0 ± 1.5, $n = 3$

[a] Cultures as examined by scoring clones 10 days after seeding in semisolid agar; 100% = 86 ± 6 clones in untreated agar cultures. [b] Colonies were scored 7−12 days following seeding; 100% = 82 ± 8.

target site, similar to the outcome of derivatization of BA with pivalic acid in **AN9**;[35] and (2) the intracellularly released ATRA and BA affect the cells synergistically.

The differentiation activity of the esters **RN1**−**RN4** in comparison with that of ATRA was determined by evaluation of NBT reduction activity of HL-60 cells after 2 days of exposure to the prodrugs (Table 2). The following order of potency: **RN1** > **RN2** > **RN3** > ATRA ∼ **RN4** was obtained. The significant differences in the relative activities of the prodrugs demonstrate that their activity depends on the specific acyl fragment attached, where the butyryl moiety was found to contribute the maximum level of activity.

The effect of **RN1** on growth of mouse lung and human pancreatic cancer cell lines was examined by colony formation assay (CFA). The comparative IC_{50} values for **RN1**, ATRA, BA, and **AN9** are given in Table 3. **RN1** is clearly superior to ATRA or BA in inhibiting growth of these cell lines. Moreover, it overcame the resistance of 3LLD122 cells to ATRA as is evident by the 7-fold decrease in IC_{50} of RN1 compared to that of ATRA (Table 3). The combination of ATRA and HDAC inhibitors was reported to overcome resistance of cells to ATRA in APL cells and in an APL patient.[24−26] Abrogating the HDAC repressive effect is not necessarily limited to the translocation-generated fusion oncogenes PML-RAR and PLZF-RAR. It is possible that in 3LLD122 cells resistant to ATRA different and yet-unidentified oncogenes repress transcription by recruiting HDAC and the BA fragment of the **RN1** relieves the repression. This supports the concept that elimination of transcription repression by HDAC inhibitors may provide a means of overcoming resistance in neoplastic diseases.[24]

Conclusions

The mechanism by which the BA prodrugs elicit their action may be mediated via inhibition of HDAC, leading to modulation in gene transcription and cellular differentiation. However, since BA as a pleotropic agent is known to elicit multiple effects,[3−5] other mechanisms of action may be involved.[36] The uniqueness of the mutual prodrug **RN1** as a potential anticancer agent stems from the combination of two moieties, BA and ATRA, each affecting a distinctive cellular target and when released simultaneously probably acting synergistically. As single agents BA and ATRA are known to possess low toxicity; therefore, their mutual prodrug is expected to exhibit low systemic toxicity.

Experimental Section

Chemistry. ^{1}H and ^{13}C NMR spectra were obtained on Bruker AM-300 and AC-200 spectrometers. Chemical shifts are expressed in ppm downfield from Me_4Si used as internal standard. Multiplicities in the ^{13}C NMR spectra were determined by off-resonance decoupling. Mass spectra (MS and HRMS) were obtained on a Finnigan 4021 spectrometer operating in CI (chemical ionization) mode. Progress of the reactions was monitored by TLC on Merck silica gel 60, 0.040−0.063 mm, eluted with hexanes−EtOAc mixtures. Preparative TLC was carried out on C18 reverse-phase Analtech HPTLC plates eluted with MeCN−water mixtures. Melting points were determined on a Fisher-Johns apparatus.

General Procedure. To a solution of ATRA (1.1 g, 3.67 mmol) and a chloromethyl ester (1.2 equiv) in dry DMF (2 mL) was added Et_3N (0.69 g, 6.8 mmol) dropwise. The product was detected by TLC (EtOAc:hexane, 1:4) as a deep yellow spot, $R_f \sim 0.7$. The solution was stirred at 70 °C for 3 h. At this point only a negligible amount of ATRA could be detected. The mixture was dissolved in ether (20 mL) and washed with brine (4 × 30 mL) and 5% $NaHCO_3$ (3 × 10 mL). The ether solution was dried ($MgSO_4$) and evaporated. The oily residue was separated on a silica gel column (ether:hexane, 1:3).

***all-trans*-Retinoyloxymethyl Butyrate, RN1.** Obtained from chloromethyl butyrate as a yellow oil (4.5 g, 68%): ^{1}H NMR ($CDCl_3$) δ 6.99 (dd, $J = 15$, 11 Hz, vinylic H, 1H), 6.30 (d, $J = 11$ Hz, vinylic H, 1H), 6.28 (d, $J = 15$ Hz, vinylic H, 1H), 6.17 (s, vinylic H, 1H), 6.11 (d, $J = 5.2$ Hz, vinylic H, 1H), 5.81 (s, OCH_2O, 2H), 5.79 (d, $J = 5.7$ Hz, vinylic H, 1H), 2.38 (d, $J = 1$ Hz, Me, 3H), 2.35 (t, CH_2CO, 2H), 2.02 (m, CH_2, 2H), 2.01 (t, Me, 3H), 1.71 (s, Me, 3H), 1.65 (m, CH_2CH_2CO, 2H), 1.45 (m, CH_2, 2H), 1.03 (s, 2 Me, 6H), 0.95 (t, Me, 3H); ^{13}C NMR ($CDCl_3$) δ 172.6, 165.4, 155.7, 140.5, 137.6, 137.2, 134.6, 132.1, 130.0, 129.3, 129.0, 116.7, 78.7, 39.6, 35.9, 34.3, 33.1, 29.0, 21.1, 19.2, 18.1, 14.1, 13.5, 12.9; MS (CI-CH_4) 401 (MH^+), 283 ($MH^+ - MeCH_2CH_2COOCH_2OH$); HRMS (CI-$CH_4$) 400.246009 ($MH^+$), calcd 400.240231, $C_{25}H_{36}O_4$. Anal. ($C_{25}H_{36}O_4 \cdot H_2O$) C, H.

***all-trans*-Retinoyloxymethyl Propionate, RN2.** Obtained from chloromethyl propionate as a yellow oil (1.18 g, 87%): ^{1}H NMR ($CDCl_3$) δ 7.05 (dd, $J = 15$, 6.5 Hz, vinylic H, 1H), 6.30 (d, $J = 16$ Hz, vinylic H, 1H), 6.29 (d, $J = 15$ Hz, vinylic H, 1H), 6.15 (d, $J = 6.5$ Hz, vinylic H, 1H), 6.14 (d, $J = 15$ Hz, vinylic H, 1H), 5.81 (s, OCH_2O, 2H), 5.79 (bs, vinylic H, 1H), 2.40 (q, $J = 7.5$ Hz, CH_2CO, 2H), 2.38 (d, $J = 1$ Hz,

Me, 3H), 2.03 (bt, $J = 6.5$ Hz, CH_2, 2H), 2.01 (s, Me, 3H), 1.71 (s, Me, 3H), 1.62, 1.47 (2 m, 2 CH_2, 4H), 1.16 (t, $J = 7.5$ Hz, 3H), 1.03 (s, 2 Me, 6H); ^{13}C NMR ($CDCl_3$) δ 173.3, 165.4, 155.7, 140.5, 137.6, 137.2, 134.6, 132.1, 129.3, 129.2, 129.0, 116.6, 78.9, 39.6, 34.2, 33.1, 28.9, 27.3, 21.7, 19.2, 14.1, 12.9, 8.7; MS (CI-NH_3) 387 (MH^+), 283; HRMS (CI-CH_4) 386.248876 (MH^+), calcd 386.245710, $C_{24}H_{34}O_4$. Anal. ($C_{24}H_{34}O_4 \cdot 0.5H_2O$) C, H.

***all-trans*-Retinoyloxymethyl Isobutyrate, RN3.** Obtained from chloromethyl isobutyrate as a yellow oil (1.44 g, 94% yield) upon evaporation of the ether solution and used without further purification. After standing in the freezer for some time, the oil partially solidified: ^{1}H NMR ($CDCl_3$) δ 7.05 (dd, $J = 15$, 11.5 Hz, vinylic H, 1H), 6.30 (d, $J = 15$ Hz, vinylic H, 1H), 6.29 (d, $J = 15$ Hz, vinylic H, 1H), 6.15 (d, $J = 11.5$ Hz, vinylic H, 1H), 6.14 (d, $J = 15$ Hz, vinylic H, 1H), 5.81 (s, OCH_2O, 2H), 5.78 (bs, vinylic H, 1H), 2.60 (septet, $J = 7$ Hz, Me_2CH, 1H), 2.38 (d, $J = 1$ Hz, Me, 3H), 2.03 (wt, $J = 6.5$ Hz, CH_2, 2H), 2.01 (s, Me, 3H), 1.71 (s, Me, 3H), 1.66, 1.47 (2 m, 2 CH_2, 4H), 1.20, 1.17 (2 s, 2 Me, 6H), 1.03 (s, 2 Me, 6H); ^{13}C NMR ($CDCl_3$) δ 176.0, 165.4, 155.5, 140.4, 137.1, 134.6, 132.0, 130.2, 129.9, 129.3, 129.1, 116.7, 78.9, 39.6, 33.8, 34.2, 33.1, 28.9, 21.7, 19.2, 18.6, 14.1, 12.9; MS (CI-NH_3) 401 (MH^+), 283 ($MH^+ - Me_2CHCOOCH_2OH$); HRMS (CI-CH_4) 400.261414 (MH^+), calcd 400.261360, $C_{25}H_{36}O_4$. Anal. ($C_{25}H_{36}O_4 \cdot 1.5H_2O$) C, H.

***all-trans*-Retinoyloxymethyl Pivalate, RN4.** Obtained from chloromethyl pivalate as a yellow oil that crystallized upon cooling in the freezer: mp 35−37 °C (1.35 g, 98% yield); ^{1}H NMR ($CDCl_3$) δ 7.04 (dd, $J = 15$, 11 Hz, vinylic H, 1H), 6.30 (d, $J = 16$ Hz, vinylic H, 1H), 6.29 (d, $J = 15$ Hz, vinylic H, 1H), 6.15 (d, $J = 11$ Hz, vinylic H, 1H), 6.14 (d, $J = 16$ Hz, vinylic H, 1H), 5.81 (s, OCH_2O, 2H), 5.78 (bs, vinylic H, 1H), 2.37 (d, $J = 1$ Hz, Me, 3H), 2.03 (bt, $J = 6.5$ Hz, CH_2, 2H), 2.01 (s Me, 3H), 1.71 (s, Me, 3H), 1.62, 1.47 (2 m, 2 CH_2, 4H), 1.22 (s, *t*-Bu, 9H), 1.03 (s, 2 Me, 6H); ^{13}C NMR ($CDCl_3$) δ 177.4, 165.3, 155.4, 140.3, 137.7, 137.2, 134.6, 131.9, 130.2, 129.3, 129.1, 116.8, 79.2, 39.6, 38.8, 34.2, 33.1, 28.9, 26.9, 21.7, 19.2, 14.1, 12.9; MS (CI-NH_3) 415 (MH^+), 299, 283; HRMS (CI-CH_4) 428.252872 (MH^+), calcd 428.256275, $C_{26}H_{38}O_4$. Anal. ($C_{26}H_{38}O_4 \cdot 1.5H_2O$) C, H.

Biology. 1. Cells Lines. 3LLD122, the highly metastatic subclone of 3LL, was obtained from L. Eisenbach (The Weizmann Institute of Science, Israel). The other cell lines were from ATCC (Rockville, MD). Cells were grown in RPM1 and 10% FCS, supplemented with 2 mM glutamine. Viability was determined by Trypan blue exclusion. Mycoplasma free cells were incubated at 37 °C in a humidified 5% CO_2 incubator.

2. Induction of Differentiation. Cancer cell differentiation was evaluated with the human leukemic cell lines HL-60 by nitro blue tetrazolium (NBT) reduction activity.[1,12] Cell cultures containing 0.1% NBT were stimulated with 0.4 μM 12-*O*-tetradecanoylphorbol 13-acetate (PMA) then incubated for 30 min at 37 °C and examined microscopically by scoring at least 200 cells. The % of NBT positive cells was calculated from the ratio the stained cells to the total cells scored and the % of positive cells in untreated culture was subtracted.

3. Inhibition of Cancer Cell Growth. (a) Formation of 3LLD122 colonies in semisolid agar bilayer: The method was based on the previously described procedure with the described modifications.[28,29] The test compounds at the indicated concentrations were added to the upper layer of agar. Aggregates of >20 cells that developed on the semisolid agar bilayer after 7 days were scored as colonies using an inverted microscope.

(b) CFA: Single-cell suspensions were plated into 60-mm tissue culture dishes at 400 $PaCa_2$ cells/dish. Following 24 h after plating the cells, drug exposure was initiated. The cultures were incubated for 7−12 days to allow the formation of colonies. Colonies were fixed with MeOH, stained with Giemsa, counted and compared to the control. All assays were performed in triplicate.

(c) Cell viability: Viability was determined by scoring under a microscope trypan blue (0.1%) excluding cells.

4. Drug Treatment. With the exception of BA which was dissolved in PBS, the drugs were dissolved in DMSO and were added to the culture at a 1:1000 dilution to give a final DMSO concentration of ≤0.1%

Acknowledgment. Generous support for this work by the Marcus Center for Pharmaceutical and Medicinal Chemistry, the Minerva Foundation for the Otto Mayerhoff Center for the Study of Drug−Receptor Interactions, the Bronia Hacker Fund for Scientific Instrumentation at Bar Ilan University, and Beacon Laboratories is gratefully acknowledged. We thank Ms. Victoria Pugach for technical assistance.

References

(1) Breitman, T.; Selonick, S.; Collins, S. Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2936−2940.
(2) Castaigne, S.; Chomienne, C.; Daniel, M. T.; Ballerini, P.; Berger, R.; Fenaux, P.; Degos, L. All-trans-retinoic acid as differentiation therapy for acute promyelocytic leukemia. I. Clinical Results. *Blood* **1990**, *76*, 1704−1709.
(3) Prasad, K. N. Butyric Acid: A small fatty acid with diverse biological functions. *Life Sci.* **1980**, *27*, 1351−1358.
(4) Kruh, J. Effects of sodium butyrate, a new pharmacological agent, on cells in culture *Mol. Cell. Biochem*. **1982**, *42*, 65−82.
(5) Rephaeli, A.; Nordenberg, J. Cytodifferentiation inducers as an anti-cancer agents. *Int. J. Oncol*. **1994**, *1*, 481−487.
(6) Mangelsdrof, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schutz, G.; Umesono, R.; Blumberg, B.; Kasmer, P.; Mark, M.; Chambon, P. The nuclear hormone receptor superfamily, the second decade. *Cell* **1995**, *83*, 835−840.
(7) Minucci, S.; Pelicci P. G. Retinoid receptors in health and disease: co-regulators and the chromatin connection. *Cell Dev. Biol.* **1999**, *10*, 215−225.
(8) Chen, H.; Lin, RJ.; Xie, W.; Wilpitz, D.; Evans, R. M. Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. *Cell* **1999**, *98*, 675−686.
(9) Davis, S. M.; Ross, J. A. Childhood cancer etiology: recent reports. *Med. Pediatr. Oncol.* **1999**, *32*, 49−52.
(10) Freeman, H. J. Effect of differing concentration of sodium butyrate in 1,2-dimethylhydrazine-induced rat intestinal neoplasis. *Gastroenterology* **1986**, *91*, 596−602.
(11) Miller, A. A.; Kurschel, E.; Osieka, R.; Schmidt, C. Clinical pharmacology of sodium butyrate in patients with acute leukemia. *Eur. J. Cancer Clin. Oncol*. **1987**, *23*, 1283−1287.
(12) Rephaeli, A.; Nordenberg, J.; Aviram, A.; Rabizadeh, E.; Zimra, Y.; Nudelman, A.; Novogrodsky, A.; Shaklai, M. Butyrate induced differentiation in leukemic myeloid cells in vitro and in-vivo studies. *Oncol. Rep.* **1994**, *1*, 481−487.
(13) Vidali, G.; Boffa, L. C.; Bradbury, E. M.; Allfrey, F. G. Butyrate suppression of histone deacetylation leads to accumulation of poly-acetylated forms of histones H3 and H4 and increased DNase I sensitivity of the associated DNA sequences. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 2239−2243.
(14) Candido, E. P.; Reeves, R.; Davie, J. R. Sodium butyrate inhibits histone deacetylation in cultured cells. *Cell* **1978**, *14*, 105−113.
(15) Chen, T. A.; Allfrey, V. G. Rapid and reversible changes in nucleosome structure accompany the activation, repression, and superinduction of murine fibroblast protooncogenes c-fos and c-myc. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5252−5256.
(16) Thorne, A. W.; Kmiciek, D. K.; Mitchelson, K.; Sautiere, P.; Crane-Robinson, C. Patterns of histone acetylation. *Eur. J. Biochem*. **1990**, *193*, 701−713.
(17) Ogryzko, V. V.; Schiltz, R. L.; Russanova, V.; Howard, B. H.; Nakatani, Y. The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* **1996**, *87*, 953−959.
(18) Wolffe, A. Sinful repression. *Nature* **1997**, *387*, 16−17.
(19) Fenrick, R.; Hiebert, S. W. Role of histone deacetylases in acute leukemia. *J. Cell Biochem. Suppl.* **1998**, *30−31*, 194−202.
(20) Lin, R. J.; Nagy, L.; Inoue, S.; Shao, W.; Miller, W. H., Jr.; Evans, R. M. Role of the histone deacetylase complex in acute promyelocytic leukemia. *Nature* **1998**, *39*, 811−814.
(21) Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J. Biol Chem*. **1990**, *265*, 17174−17179.
(22) Samid, D.; Hudgins, W. R.; Shacks, S.; Liu, L.; Prasanna, P.; Myets, C. E. Phenylacetate and phenylbutyrate as novel, nontoxic differentiation inducers. *Adv. Exp. Med. Biol.* **1997**, *400A*, 501−505.
(23) Lea, M. A.; Randolph, V. M.; Hodge, S. K. Induction of histone acetylation and growth regulation in erythroleukemia cells by 4-phenylbutyrate and structural analogues. *Anticancer Res.* **1999,** *19*, 1971−1976.

(24) Warrell, R. P., Jr.; He, L. Z.; Richon, V.; Calleja, E.; Pandolfi, P. P. Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase. *J. Natl. Cancer Inst.* **1999**, *90*, 1621−1625.
(25) Grignani, F.; De Matteis, S.; Nervi, C.; Tomassoni, L.; Gelmetti, V.; Cioce Fanelli, M.; Ruthardt, M.; Ferrara, F. F.; Zamir, I.; Seiser, C.; Grignani, F.; Lazar, M. A.; Minucci, S.; Pelicci, P. G. Fusion proteins of the retinoic acid receptor alfa recruit histone deacetylase in promyelocytic leukemia. *Nature* **1998**, *391*, 815−816.
(26) He, L. Z.; Guidez, F.; Tribioli, C.; Peruzzi, D.; Ruthardt, M.; Zelent, A.; Pandolfi, P. P. Distinct interaction of PML-RAR alfa and PLZF-RAR alfa in co-repressors determine differential responses to RA. *Appl. Nat. Genet.* **1998**, *18*, 126−135.
(27) Taimi, M.; Chen, Z. X.; Breitman, T. R. Potentiation of retinoic acid-induced differentiation of human acute promyelocytic leukemia NB4 cells by butyric acid, tributyrin, and hexamethylene bisactamide. *Oncol. Res.* **1998**, *10*, 75−84.
(28) Nudelman, A.; Shaklai, M.; Aviram, A.; Rabizadeh, E.; Zimra, Y.; Ruse, M.; Rephaeli, A. Novel anticancer prodrugs of butyric acid. *J. Med. Chem.* **1992**, *35*, 6876−6894.
(29) Rephaeli, A.; Shaklai, M.; Ruse, M.; Nudelman, A. Derivatives of butyric acid as potential anti-neoplastic agents. *Int. J. Cancer.* **1991**, *49*, 66−72.
(30) Aviram, A.; Zimra, Y.; Shaklai, M.; Nudelman, A.; Rephaeli, A. Comparison between the effect of butyric acid and its prodrugs pivaloyxmethyl butyrate on hyperactylation of histones in HL-60 cell lines. *Int. J. Cancer* **1994**, *56*, 906−909.
(31) Rabizadeh, E.; Shaklai, M.; Eisenbach, L.; Nudelman, A.; Rephaeli, A. Esterase inhibitors diminish the modulation of gene expression by butyric butyric acid derivative, pivaloyloxymethyl butyrate (**AN-9**). *Israel J. Med. Sci.* **1996**, *32*, 1186−1191.
(32) Zimra, Y.; Wasserman, L.; Maron, L.; Shaklai, M.; Rephaeli, A.; Nudelman, A. Butyric acid and pivaloyloxymethyl butyrate, **AN-9**, a novel butyric acid derivative, induce apoptosis in HL-60 cells. *J. Cancer Res. Clin. Oncol.* **1997**, *123*, 152−160.
(33) Bundgaard, H. R.; Nielsen, N. M. Esters of *N*,*N*-disubstituted 2-hydroxyacetamides as novel highly biolabile prodrug type for carboxylic acid agents. *J. Med. Chem.* **1987**, *30*, 451−455.
(34) Eckhart S. D.; Villaona-Calero, M. A.; Hommond, L. A phase I study of **AN-9**, pivaloyloxymethyl butyrate (Pivanex) a lipophilic butyric acid analogue, in patients with advanced solid tumors. *Proc. AACR* **1988**, *80*, #3449.
(35) Zimra, Y.; Maron, L.; Shaklai, M.; Nudelman, A.; Rephaeli, A. Pivaloyloxymethyl butyrate (AN-9) exhibits higher anticancer activity than butyric acid (BA): It penetrates faster than the acid and induces apoptosis in HL-60 cells. *Blood* **1994**, *84*, 10.
(36) Warrel, R. P.; Samid, D. Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase. *J. Natl. Cancer Inst.* **1999**, *91*, 475−476.

JM990540A