Communications to the Editor

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DIFFERENTIATION INDUCERS OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS HL-60. PHENYLCARBAMOYLBENZOIC ACIDS AND POLYENE AMIDES

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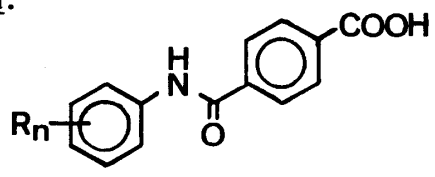
New inducers of the differentiation of human promyelocytic leukemia cells HL-60 to mature granulocytes, 4-(3,4-diisopropylphenylcarbamoyl)benzoic acid (2c) and 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthyl-2-carbamoyl)benzoic acid (2d), have been found. Two polyene amides which are structural hybrids of retinoic acid and the amide compounds 1a and 2d also exhibited the biological activity, and this result suggested a structural link between retinoic acid and the active aromatic amides.

KEYWORDS—— differentiation; phenylcarbamoylbenzoic acid; retinoic acid; polyene amide; retinoid; HL-60; leukemia

We have reported that terephthalic anilides (1),[1] e.g. Am 80 (1a), have strong activity to induce the differentiation of human promyelocytic leukemia cells HL-60 to mature granulocytes. In the course of the study, we became interested in the activity of compounds (2) in which the amide bond of 1 is reversed: the electronic nature of the two benzene rings of 2 must be very different from that of 1. This paper describes the new amide compounds, which show strong inducing activity, and some hybrid compounds of retinoic acid and the amide compoud 1a and 2d.



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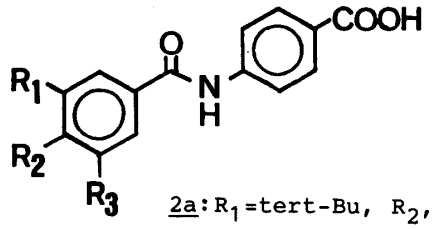
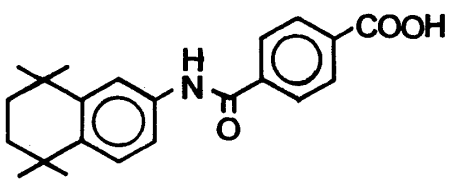
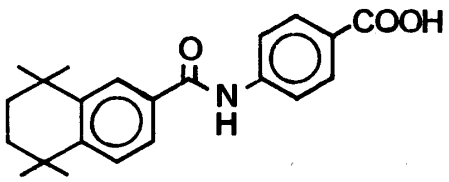


2a: R_1=tert-Bu, R_2,R_3=H
2b: R_1,R_3=tert-Bu, R_2=H
2c: R_1,R_2=iso-Pr, R_3=H



1a (Am 80)



2d

The amide compounds (2) were prepared by condensation of a substituted benzoyl chloride and methyl 4-aminobenzoate, followed by alkaline hydrolysis. The melting points are shown in the Table I. The hybrid compound (3) was prepared by the condensation of a carboxylic acid derived from ionone (by oxidation with sodium hypochlorite) and methyl 4-aminobenzoate, followed by alkaline hydrolysis. The hybrid compound (4) was prepared by the condensation of 2-amino-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene (6) and muconic acid monomethyl ester. An analogous compound (5) was prepared from 6 and fumaric acid monomethyl ester.

The differentiation-inducing activity of HL-60 cells was determined morphologically by examination under a microscope after Wright-Giemsa staining, and functionally by measuring Nitroblue tetrazolium (NBT) reduction in the presence of 12-O-tetradecanoylphorbol-13-acetate (TPA),[3] according to the previous papers.[1,4] The degree of differentiation was examined after incubation for 4 days.

Table I. Differentiation of HL-60 Cells after Incubation with Phenylcarbamoylbenzoic Acids

Compounds	mp (°C)	log M	Myeloid cell type,[a] % A	B	C	NBT-positive[b] cells, %
Control			98	2	0	2
Retinoic acid		-8	15	30	55	61
		-9	66	20	14	19
		-10	94	6	0	3
2a	232-234	-6	78	18	4	12
		-7	91	7	2	3
2b	265-266	-6	27	35	38	40
		-7	41	35	24	37
		-8	55	24	21	32
		-9	72	20	8	16
		-10	94	6	0	7
2c	223-224	-6	32	28	40	46
		-7	41	27	32	44
		-8	56	26	18	27
		-9	84	15	2	9
		-10	98	2	0	2
2d	265-267	-7	26	31	43	53
		-8	40	23	37	56
		-9	38	36	27	53
		-10	82	10	8	18
		-11	99	1	0	6
3	235-237	-6	40	47	13	78
		-7	65	27	7	56
4	236.5-237	-6	7	66	27	88
		-7	43	45	12	67

a) A, promyelocytes; B, myelocytes and metamyelocytes; C, banded and segmented neutrophiles.

b) The percentage of cells containing formazan.

The differentiation-inducing activities of 2a-d are quite similar to those of the corresponding 1, though the electronic properties such as pKa of the carboxylic acid and the charge distribution of the new amides 2a-d should be very different from those of the other amides 1. The morphological changes clearly showed the induction of mature granulocytes, myelocytes, metamyelocytes and neutrophiles. The ratio of NBT-reducing cells paralleled the results of the morphological assessment. The data in Table I are representative examples from more than two experiments. The substituent effect on the left benzene ring of 2 is striking and parallel to the effect seen in the series of 1. Thus, the bulky alkyl substituent at the meta position is the most important (2a, 2b). 4-(3,4-Diisopropyl phenylcarbamoyl)benzoic acid (2c) is as active as retinoic acid, and 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthyl-2-carbamoyl) benzoic acid (2d) is more active than retinoic acid and as active as Am 80 (1a). The methyl esters are 1/10 as active as the corresponding free acids. These results suggest that the structure of the group intervening between the two phenyl groups can be varied over a wide range regardless of its electronic effect. This hypothesis is supported by the observation of inducing activity in compounds where the intervening group is $-SO_2NH-$, -CO-O-, -O-CO-, or others (data not shown), in addition to the reported -N=N- and -CH=CH-.[4] These groups may have a role in determining a certain steric conformation between the polar carboxylic acid group and the hydrophobic alkyl substituent on the phenyl ring.

Since the structures of these amide compounds (1 and 2) seem superficially to differ from that of retinoic acid, the hybrid compounds of retinoic acid and the amide compounds attracted our interest (Chart 1). Compound 3 is constructed from the left half of retinoic acid and the right half of 2d, and compound 4 from the right half of retinoic acid and the left half of 1a. Compoud 5 is a nor-acid of 4. The differentiation-inducing activities of 3 and 4 were found to be about 1/10 of that of retinoic acid, each leading the HL-60 cells to mature granulocytes (Fig. 1). The fumaric amide 5 was weaker than 3 and 4, as is the case with (E,E,E)-5-

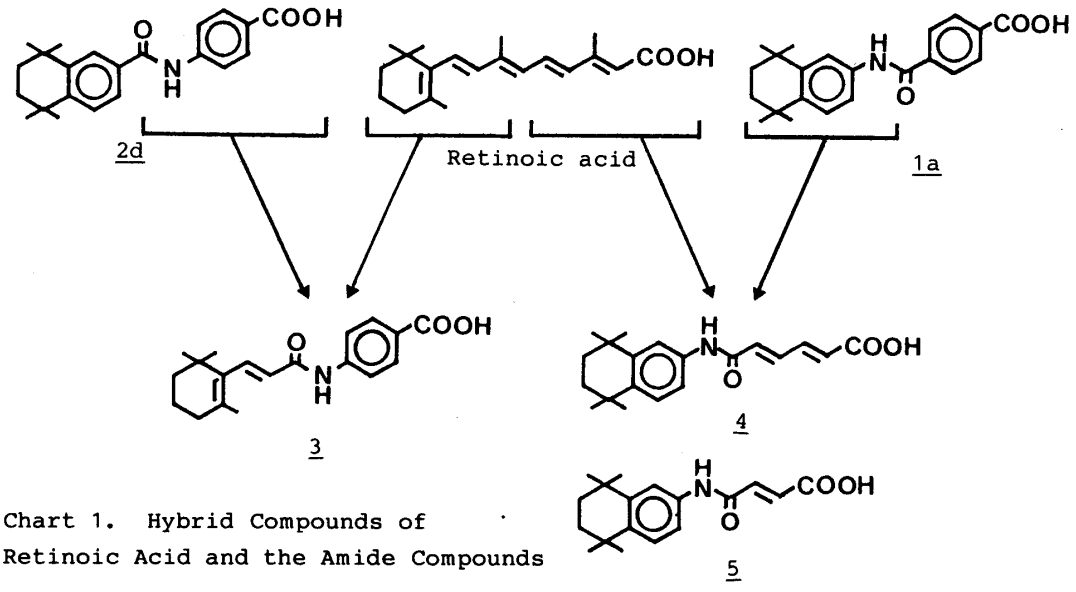


Chart 1. Hybrid Compounds of Retinoic Acid and the Amide Compounds

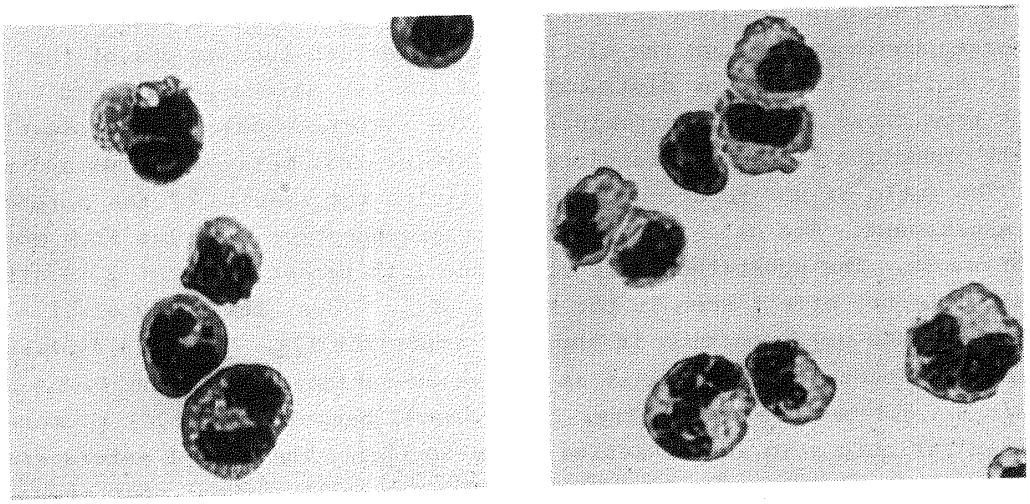


Fig. 1. Morphology of Induced HL-60 Cells Cultured in the Presence of 3 and 4 for 4 Days

Cytospin slide preparations of suspension cell cultures stained with Wright-Giemsa (x 400). Cells in this figure consist of metamyelocytes and banded neutrophils. Left: 3 (10^{-6} M), Right: 4 (10^{-6} M).

methyl-7-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-heptatrienoic acid (13,14-dinorretinoic acid).[5] The significant activity of the hybrid compounds strongly supports the idea that these amide compounds and retinoic acid are structurally related agonists. Further, the structure-activity relationships of azobenzene-carboxylic acids and stilbenecarboxylic acids also support this conclusion.[4]

These findings may constitute a breakthrough in the search for new retinoidal active substances, which may be clinically useful in oncology and dermatology.[6]

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