Communications to the Editor

[Chem. Pharm. Bull. 33(1) 404—407 (1985)]

Chalcone Carboxylic Acids. Potent Differentiation Inducers of Human Promyelocytic Cells HL-60

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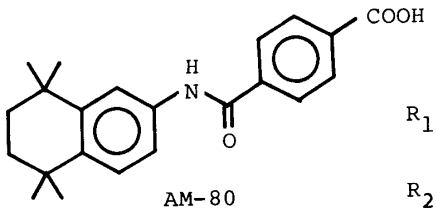
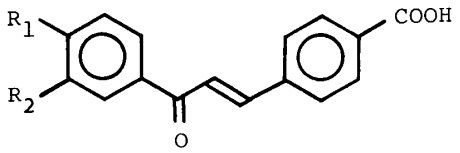
Alkyl-substituted chalcone-4'-carboxylic acids are very potent inducers of differentiation of human promyelocytic cells HL-60. The differentiated cells are morphologically and functionally granulocytes.

KEYWORDS —— chalcone-4'-carboxylic acid; myelocyte; HL-60; leukemia; differentiation

Retinoids (retinoic acid, and its natural and synthetic analogs) affect the normal differentiation of epethelium.[1] Certain of these compounds inhibit the growth of transformed cells.[2] The antineoplastic effects of retinoids suggest that these compounds could be useful therapeutically and for the chemoprevention of cancer.[3] Another important activity of retinoids is the induction of terminal differentiation of human promyelocytic leukemia cells (HL-60) to granulocytes.[4] Because acute myeloid leukemia can be looked upon as a disease involving a block in differentiation, therapeutic application of differentiation-inducing agents is very promising. However, specific inducers such as retinoids are too toxic to be used clinically. Thus, we have been searching for new potent inducers of cell differentiation and for antagonists of tumor promoters. Recently we found a very potent inducer of granulocyte-differentiation, a terephthalic anilide (AM-80).[5] Subsequently, we designed and prepared several chalcone derivatives, and some of them were found to be strikingly potent inducers of differentiation of HL-60 cells to granulocytes.



AM-80



1 R_1=H, R_2=H
2 R_1=t-Bu, R_2=H
3 R_1=iso-Pr, R_2=iso-Pr

The HL-60 cell line has been maintained in suspension culture[6] in plastic flasks in RPMI-1640 medium supplemented with 5% fetal calf serum and antibiotics (penicillin G and streptomycin). Cells were cultured with inducers for 4 days, and stained with Wright-Giemsa. Differential counts were then performed on a

minimum of 200 cells under a light microscope. Nitroblue tetrazolium (NBT) reduction was assayed as described.[7] Cells were incubated for 20 min at 37°C with an equal volume of phosphate-buffered saline containing tetradodecanoylphorbol 13-acetate (200 ng/ml). The percentage of cells containing blue-black formazan was determined. Chalcone carboxylic acids were prepared from acetophenone derivatives and terephthalic aldehyde methyl ester in the presence of a base such as potassium hydroxide or potassium bicarbonate, followed by mild hydrolysis with aqueous alkali. The melting points are shown in Table I.

The parent compound, chalcone 4'-carboxylic acid (1), was completely inactive at concentrations ranging from 10^{-6} to 10^{-9} M. However, 4-tert-butyl-chalcone 4'-carboxylic acid (2) exhibited a potent inducing activity of HL-60 cells to granulocytes. The activity was observed even at a concentration as low as 10^{-8} M (Table I). The positive control (retinoic acid) showed the activity at concentrations above 10^{-9} M. The differentiated cells induced by 2 are mature, having morphological characteristics of myelocytes and metamyelocytes, and some were banded and segmented neutrophils. Table I summarizes the morphological differentiation results with some of the chalcone derivatives, which are essentially reproducible.

Table I. Differentiation of HL-60 Cells after Incubation with Chalcone-4'-carboxylic Acids and Esters

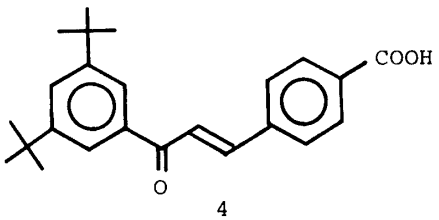
Comp.[a]	mp (°C)	log M	Myeloid cell type,[b] %			NBT-positive[c] cells, %
			A	B	C	
Control			94	6	0	7
Retinoic acid		-8	11	73	16	52
		-9	43	46	10	32
		-10	89	11	0	11
1	224-225.5	-6	95	5	0.5	7
2	245-246	-7	18	76	7	69
		-8	82	17	1	18
2a	119-120.5	-6	86	14	0.5	14
3	197.5-199	-8	10	69	20	59
		-9	65	32	3	33
		-10	90	10	0.5	12
4	202-203.5	-8	3	51	46	53
		-9	7	64	29	61
		-10	27	51	22	37
		-11	93	6	0.4	16
5	203-204	-8	9	71	19	71
		-9	59	35	6	51
		-10	78	18	4	22
		-11	94	6	0	8
5a	93.5-94	-7	16	64	20	55
		-8	75	21	3	14
		-9	99	1	0	13

a) 2a and 5a are the methyl esters of 2 and 5, respectively.

b) A, promyelocytes; B, myelocytes and metamyelocytes; C, banded and segmented neutrophils.

c) The percentage of cells containing formazan.

The ability of cells to reduce NBT is a good marker for differentiation.[7] This differentiation-associated assay showed that the effect of the chalcone derivatives is the induction of granulocyte differentiation. The NBT-reducing activity of 2 was evident even at 10^{-8} M (Table I). The dose-response relationship is very well correlated with the morphological changes.



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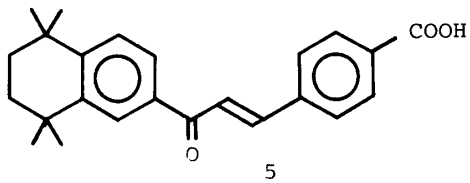

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A bulky hydrophobic group on the phenyl ring (on the carbonyl side) of the chalcone skeleton seems to be preferable for the inducing activity: 3,4-di-iso-propylchalcone (3) and 3,5-di-tert-butylchalcone (4) are more active than 2. The morphological and functional changes characteristic of myeloid differentiation were observed at 10^{-9} M 3 and 10^{-10} M 4 (Table I and Fig. 1). Figure 2 illustrates the mature cells induced by 4. These compounds are definitely more active than trans- retinoic acid. Tetramethyl-3,4-tetramethylenechalcone carboxylic acid (5) is also very active.

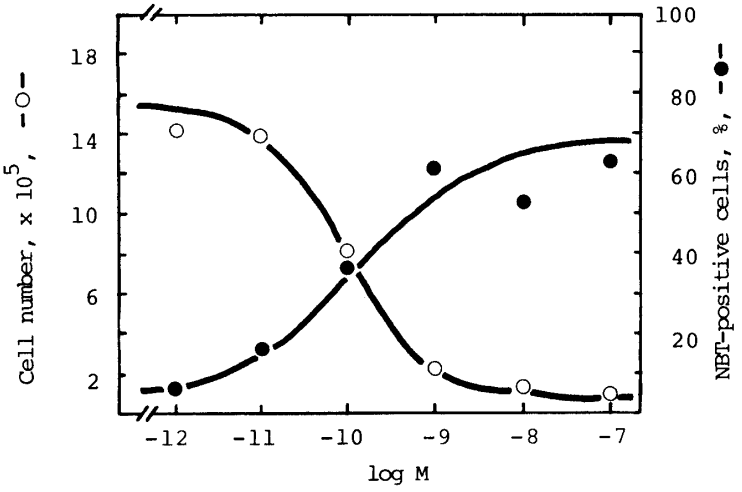


Fig. 1. Dose-Response Curves for NBT Reduction Assay and for Cell Proliferation in the Presence of 3,5-Di-tert-butylchalcone-4'-carboxylic acid (4)

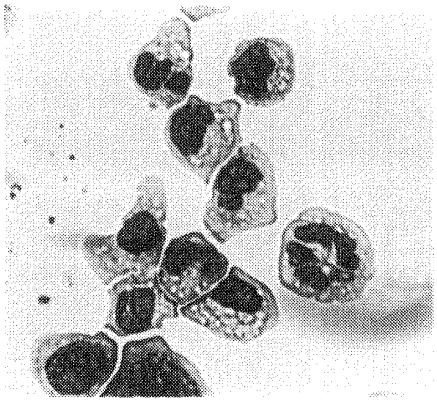


Fig. 2. Morphology of Induced HL-60 Cells Cultured in 10^{-8} M for 4 Days

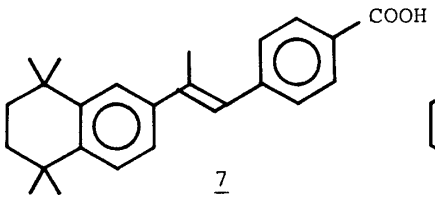
Cytopsin slide preparations of suspension cell cultures stained with Wright-Giemsa (x 400). Cells illustrated consist of metamyelocytes and banded neutraphils.

As observed in AM-80, cell growth in the presence of these chalcone carboxylic

acids ceased by day 4, although most of the cells were viable. (It may be worth noting that the pH of the medium incubated in the presence of the active chalcones did not decrease). This indicates that these chalcone derivatives induce terminal differentiation to cells which cannot proliferate further.

Methyl esters (2a, 5a) of the acids (2, 5) are about two orders of magnitude less active than the corresponding carboxylic acids. A carboxamide (6) corresponding to 5 exhibited activity at 10^{-6} - 10^{-7} M. Replacement of the 4'-carboxylic acid group with an alkyl, a halogen or a hydroxyalkyl group abolished the activity (data not shown). Transfer of the carboxylic acid group to the 3' position retained the inducing activity (effective at 10^{-6} M, data not shown).

It might be interesting to compare the chalcone derivatives with a stilbene derivative (7) and trans- and cis-retinoic acids.[4] The tert-butyl group of the chalcone 4, and the dimethyl groups on the tetrahydronaphthalene ring of 5 and the stilbene 7[8] superficially correspond to the dimethyl group on the cyclohexene ring of retinoic acids. It seems to be important that the carboxylic acid moiety is located at a constant distance from the lipophilic bulky groups. We also speculate that these bulky groups may correspond to the aliphatic hydrophobic group of teleocidin B[9] or AM-80.[5] We need more examples in order to estimate the importance of the alkyl group more reliably.



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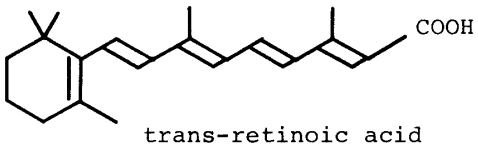

trans-retinoic acid

Since the present chalcones are strong inducers, it may be possible to develop them for clinical use. At the same time we are planning to synthesize a conformationally more rigid compound which might be useful as a probe for receptor mapping. Biochemically, the binding of the present chalcone and AM-80 to the retinoid-binding protein will be investigated.

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(Received October 6, 1984)