SYNERGISTS FOR RETINOID IN CELLULAR DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS HL-60

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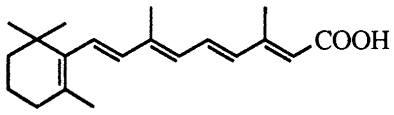
4-[5*H*-2,3-(2,5-Dimethyl-2,5-hexano)-5-methyldibenzo[*b*, *e*]diazepin-11-yl]benzoic acid (**4**) enhanced the differentiation-inducing activity of retinoic acid (**1**) and of a synthetic retinoid Am80 (**2**) toward human promyelocytic leukemia cells HL-60, although **4** alone did not induce differentiation. The synergistic effect of **4** on the activities of retinoids was also seen in suppression of proliferation of HL-60 cells.

KEY WORDS retinoid; retinoic acid; synergist; dibenzodiazepine

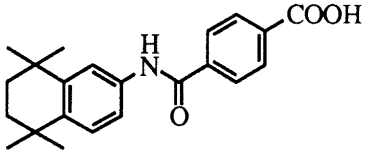
Retinoids, all-*trans*-retinoic acid (**1**) and its analogs, have significant roles in cell differentiation, proliferation and embryonic development.[1] Retinoic acid (**1**) has important clinical applications[2] in the treatment of proliferative dermatological diseases and leukemia[3] and in the prevention of some tumors. Such biological activities are mediated by binding to and activation of the specific retinoic acid nuclear receptors (RARs), followed by modulation of target gene transcription.[4] There exists another set of specific nuclear receptors (RXRs), which bind only weakly to all-*trans*-retinoic acid (**1**), but strongly to its geometrical isomer, 9-*cis*-retinoic acid.[5] Therefore, elucidation of the behaviors of such receptors is significant for understanding retinoidal action.

Previously, we reported several retinoid antagonists, based on the ligand superfamily concept.[6,7] The dibenzodiazepine derivative LE135 (**3**) inhibited the cellular differentiation of human promyelocytic leukemia cells HL-60 induced by retinoic acid (**1**) or the potent synthetic retinoid Am80 (**2**).[8,9] During an investigation on the structure-activity relationships of retinoid antagonists, we found 4-[5*H*-2,3-(2,5-dimethyl-2,5-hexano)-5-methyldibenzo[*b*, *e*]diazepin-11-yl]benzoic acid (**4**) and 4-(5*H*-2,3-diisopropyl-5-methyldibenzo[*b*, *e*]diazepin-11-yl)benzoic acid (**5**) which strongly

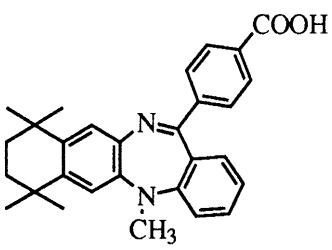
Fig. 1



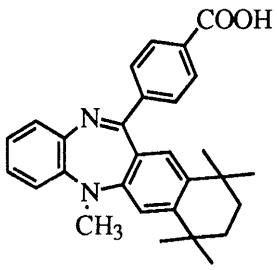
retinoic acid (all-*trans*, **1**)



Am80 (**2**)
agonistic



LE135 (**3**)
antagonistic



HX600 (**4**)
synergistic

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enhanced the activity of retinoic acid (**1**) or Am80 (**2**) in HL-60 assay although they were inactive by themselves.

The dibenzodiazepine derivative **4** (designated as HX600) was synthesized similarly to LE135 (**3**)[7] and was obtained as orange crystals (mp 282 °C; Anal $C_{29}H_{30}N_2O_2$). It is an isomer of the retinoid antagonist LE135 (**3**). We examined its ability to induce differentiation of HL-60 cells[10] to mature granulocytes,[11] which correlates well with other retinoidal activities.[8,9] Compound **4** was absolutely inactive in the induction of cellular differentiation of HL-60 cells below 10^{-6} M, as judged from morphology and biochemical nitro blue tetrazolium (NBT) reduction assay,[12] as was LE135 (**3**). Thus, we examined the effect of **4** on the differentiation of HL-60 cells induced by retinoids. In contrast to the antagonistic activity of LE135 (**3**), **4** enhanced the activity of retinoic acid (Fig. 2a). For example, the response to retinoic acid (**1**) at 10^{-8} M (54 % of NBT-positive cells) or 3.3×10^{-9} M (36 %) was increased more than 90 % by the addition of 3.3×10^{-7} M **4**. The activity of Am80 was also enhanced by the addition of **4** (Fig. 2b). The percentage of differentiated cells caused by 1×10^{-9} M Am80 (44 %) was increased to 48 %, 65 %, 90 % and 93 % in the presence of 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, and 1×10^{-6} M **4**, respectively. The effects of **4** on the differentiation-inducing activities of retinoic acid or Am80 are dose-dependent. Similarly, the addition of **4** affected the growth of HL-60 cells. Compound **4** alone (10^{-6} M) did not significantly inhibit the cell growth. The percentage of growth inhibition by retinoic acid (31 % at 10^{-9} M, and 52 % at 3.3×10^{-9} M) was increased 84 % and 93 %, respectively, by the addition of 3.3×10^{-7} M **4**. Similar effects were observed in the case of Am80 (**2**, Fig. 2b). Compound **5**, having two isopropyl groups instead of the cyclic alkyl group (i.e. the 2,5-dimethyl-2,5-hexano group of **4**), also showed a similar synergistic activity at concentrations of more than 10^{-7} M. Compound **6** without alkyl group on the benzene ring did not show synergistic or antagonistic activity.

Generally, retinoids are considered to act as inducing factors for the nuclear receptors RARs and/or RXRs, and heterodimers of RAR and RXR play major roles in retinoidal actions.[4] Retinoic acid (**1**) binds and activates RARs, but not RXRs, and would isomerize to 9-*cis* form to bind RXRs.[5] On the other hand, Am80 and a number of related compounds (so-called retinobenzoic acids[9]) bind only RARs. From this result and studies using receptor-selective retinoids,[13] the

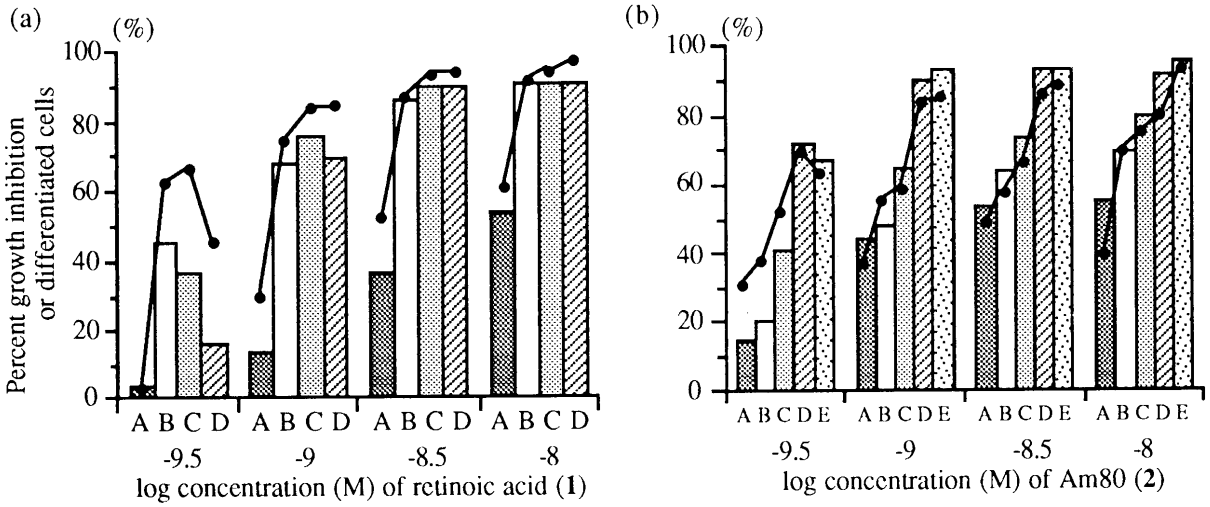

Fig. 2. (a) Synergistic Effect of **4** with Retinoic Acid (**1**) on Growth Inhibition (Lines) and Diffferentiation-Inducing Activity (Bars) in HL-60 Cells. A: **1** only ; B: **1** + 1.1×10^{-7} M **4** ; C: **1** + 3.3×10^{-7} M **4** ; D: **1** + 1.0×10^{-6} M **4** . (b) The Same Experiment for **4** and Am80 (**2**). A: **2** only ; B: **2** + 1.0×10^{-9} M **4** ; C: **2** + 1.0×10^{-8} M **4** ; D: **2** + 1.0×10^{-7} M **4** ; E: **2** + 1.0×10^{-6} M **4**.

binding of retinoids to RARs, not to RXRs, is critical for the RAR-RXR heterodimers to elicit various retinoidal activities. At present, the mechanism of the remarkable synergistic effect of **4** with retinoids is unclear. One possibility is that **4** binds to RXR and activates RAR-RXR heterodimers. Most synthetic RXR-ligands so far known show weak RXR affinities or RXR-dependent biological activities.[14] Dawson et al. reported that the combination of an RAR-specific ligand with an RXR-specific ligand did not show any synergistic effect in myeloid cell differentiation.[13] Various apparently retinoid-synergistic compounds, such as interferons, dibutyryl cAMP, barbiturates, fatty acids, or hemins, have been reported,[15] but most of them are nonspecific (high dose) or affect some cell condition. Considering the structure of **4** (and **5**), the present synergistic effect may be specific event in the nucleus. Further mechanistic investigations, including evaluation of the receptor-binding affinities of **4**, are in progress.

In conclusion, the dibenzodiazepine derivative **4** strongly increased the ability of retinoids to induce differentiation of HL-60 cells. This synergistic retinoid **4** may be a useful tool for the elucidation of retinoidal actions. Furthermore, considering the high toxicities of retinoic acid and other retinoids, **4** may have potential in the clinical applications of retinoids.

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