Retinobenzoic Acids. 6. Retinoid Antagonists with a Heterocyclic Ring

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Received September 7, 1993®

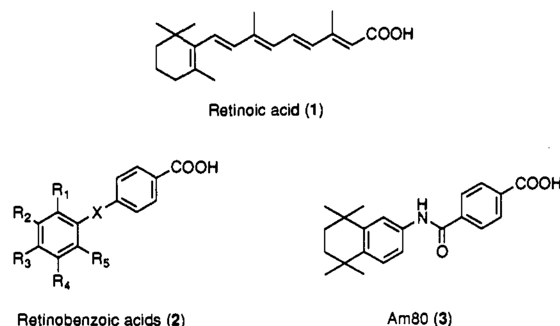
Several candidate retinoid antagonists were designed on the basis of the ligand superfamily concept and synthesized. Retinoidal activities of these benzimidazole and benzodiazepine derivatives were examined by assay of differentiation-inducing activity on human promyelocytic leukemia cell line HL-60. The parent benzimidazole derivative, 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphth-[2,3-*d*]imidazol-2-yl)benzoic acid (**7a**), and related compounds with a small alkyl group instead of the hydrogen on the nitrogen (^{1}N) atom of the imidazole ring exhibited retinoidal activity, and the potency strongly depended on the bulkiness of the substituent. The compounds having a phenyl or benzyl group on the nitrogen lacked differentiation-inducing activity on HL-60 cells and acted as antagonists to the potent retinoid 4-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carbamoyl]benzoic acid (Am80). Among the compounds possessing a seven-membered heterocyclic ring as a linking group, 4-(5*H*-7,8,9,10-tetrahydro-5,7,7,10,10-pentamethylbenzo[*e*]-naphtho[2,3-*b*][1,4]diazepin-13-yl)benzoic acid (**16**) also exhibited the antagonistic activity. The binding abilities of these compounds to retinoic acid receptors α and β were consistent with their potency for the inhibition of HL-60 cell differentiation induced by the retinoid Am80.

Introduction

Retinoic acid (*all-trans*, **1**) has a broad spectrum of biological activities involving cellular differentiation and proliferation[1] and is essential for normal embryonic development in vertebrates.[2] These biological responses are mediated by binding to and activation of the specific retinoic acid receptors (RARs)[3,4] followed by modulation of target gene transcription by the complex.[5] Clinical applications of retinoic acid (**1**) have had some success in the treatment of proliferative dermatological diseases,[6] leukemia, and some tumors.[7] Consequently, a number of derivatives of retinoic acid (**1**), called retinoids as a biological general term,[8] have been synthesized, and their therapeutic potencies have been investigated.[9] Previously, we reported that various types of benzoic acids (general formula, **2**) exhibited strong retinoidal activities, and we named them retinobenzoic acids.[10] 4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carbamoyl]benzoic acid (Am80, **3**) is a typical retinobenzoic acid,[11] exhibiting higher retinoidal activity and lower toxicity than retinoic acid (**1**) or other conventional retinoids.[12] The biological activities of retinobenzoic acids were confirmed to result from interactions with the RARs.[13,14]

RARs belong to the steroid/thyroid nuclear receptor superfamily[15] and have three subtypes: RAR α, β, and γ.[3,5] Furthermore, recent biological studies have revealed that the receptor superfamily includes another set of specific retinoid receptors, RXR α, β, and γ, that do not bind *all-trans*-retinoic acid (**1**) but do bind its geometrical isomer, 9-*cis*-retinoic acid.[16] The complexes of RARs or RXRs with their ligand form homo- or heterodimers, and elucidation of the behavior of each of the receptors and their dimers is one of the major current problems in the field of retinoidal action. Therefore, compounds that possess specific binding properties, that is, receptor-selective retinoids[17,18] or retinoid antagonists,[19] should be very useful tools for the precise elucidation of the mechanisms of retinoidal actions and also might enhance the clinical utility of retinoids by allowing separation of the wide variety of retinoid-mediated biological phenomena. In the course of investigating the structure–activity relationships of retinobenzoic acids, we found retinoid antagonists having a bulky pentacyclotetradecane (diamantyl) group at the hydrophobic area (R_2 in the general formula **2**).[20] Recently, our further investigations based on the ligand superfamily concept resulted in the development of *N*-substituted benzimidazolylbenzoic acids (**7e** and **7f**) as retinoid antagonists of a different type.[21] In this article, the design, synthesis, and biological activities of various benzimidazole derivatives and another heterocyclic antagonist are reported.

Chart 1



Design and Chemistry

Recent investigations on small molecules that control specific biological phenomena, such as steroid hormones, thyroid hormone, vitamin D_3, and so on, have shown that these molecules possess their own specific nuclear receptors which make up a group called the steroid/thyroid nuclear receptor superfamily.[15] Retinoic acid receptors (RARs) and another recently discovered group of retinoid receptors (RXRs) belong to this receptor superfamily.[5] Since the receptors in the superfamily are assumed to have evolved

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® Abstract published in *Advance ACS Abstracts*, April 1, 1994.

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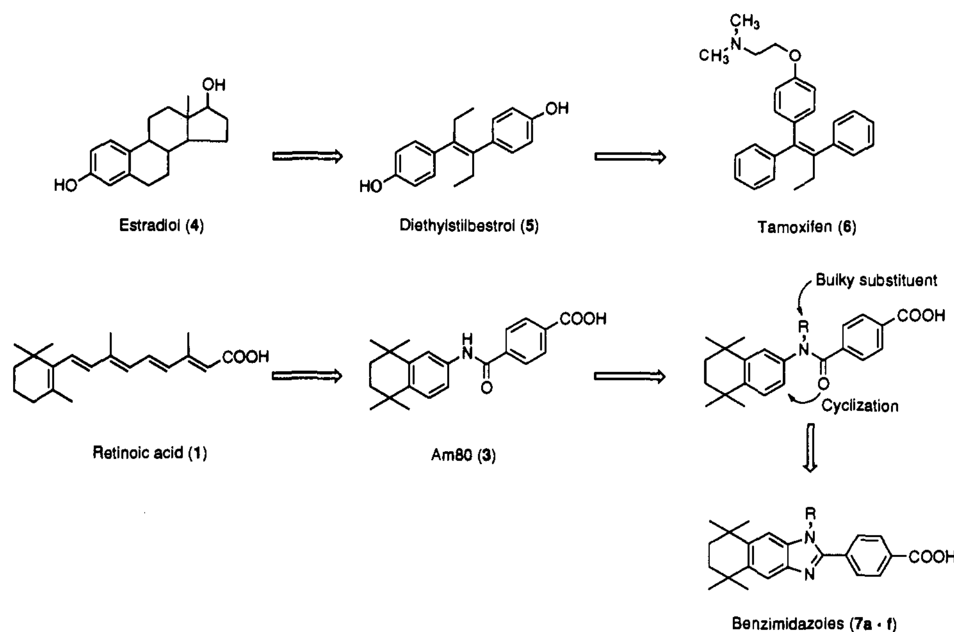
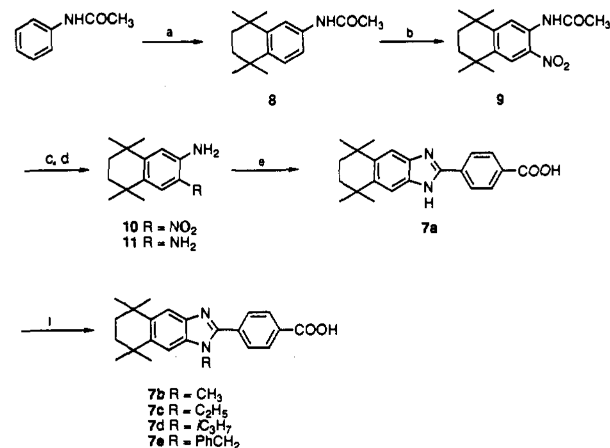
Chart 2



from a single gene,[22] the structures of their ligands (or in other words, the shapes of the ligand-binding pockets of the receptors) are possibly related to each other. On the basis of this ligand superfamily concept,[21] we have designed retinoid antagonists by applying the structure–activity relationships of estrogen agonists/antagonists to retinoids, as shown in Chart 2. The structural requirements for potent antagonists of estradiol (4) and diethylstilbestrol (5) may possibly also apply to antagonists of retinoic acid and the retinoid agonist Am80 (3) or other retinobenzoic acids. The structures of some synthetic estrogens and retinoids are significantly related each other, since in both cases these agonists contain two phenyl groups bearing the important functional groups. Indeed, various derivatives of diethylstilbestrol can be generalized by the structure corresponding to 2.[23] The introduction of a [(dimethylamino)ethoxy]phenyl group on the linking group (C=C) of diethylstilbestrol generated the estrogen antagonist, tamoxifen (6). If this structural modification of estrogen agonist to antagonist can be applied to the structures of retinoid agonists, introduction of a bulky substituent on the linking group of Am80 (3), that is the amide group, might generate a retinoid antagonist. Structure–activity studies on retinoidal amides including Am80, however, have shown that the introduction of a substituent on the amide nitrogen atom of Am80 caused a drastic conformational change of the amide bond from *trans* to *cis*, with disappearance of the retinoidal activity.[24] Therefore, in order to prevent a conformational change of the linking group by the introduction of a bulky group, the conformation which is restricted by a ring system was designed. The amide bond in Am80 could be replaced by a (benz)imidazole ring, and then a bulky substituent was introduced on the imidazole nitrogen atom. Thus, benzimidazole derivatives such as 7 were synthesized as candidate retinoid antagonists starting from the structures of Am80 and tamoxifen. In order to confirm the retinoidal activity of the parent benzimidazolylbenzoic acid, several compounds (7a–c) with a small substituent on the imidazole nitrogen atom were also synthesized.

The parent benzimidazole derivative 7a was prepared as shown in Scheme 1. Friedel–Craft alkylation of

Scheme 1[a]

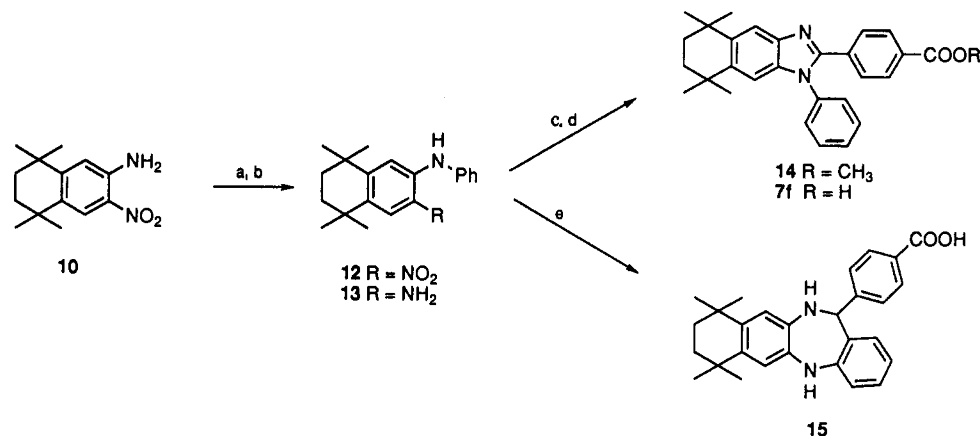


[a] (a) 2,5-Dichloro-2,5-dimethylhexane/$AlCl_3$; (b) KNO_3/CF_3SO_3H; (c) NaOH/EtOH; (d) Fe/HCl; (e) *p*-OHC-Ph-COOH; (f) KOH/CH_3I for 7b; NaH/C_2H_5I for 7c; NaH/C_3H_7I for 7d; $NaH/PhCH_2Br$ for 7e.

acetanilide in methylene chloride at 0 °C gave 6-acetamido-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (8) in 55%. 8 was nitrated, followed by hydrolysis, to afford 5,6,7,8-tetramethyl-5,5,8,8-tetrahydro-3-nitro-2-naphthylamine (10), which was reduced to the diamine (11). Condensation of phenylenediamine and aromatic carboxylic acids usually affords 2-arylbenzimidazoles in poor yield, and indeed the reaction of 11 with terephthalic acid monomethyl ester gave only trace amounts of benzimidazole 7a and its methyl ester. It was reported that the imine obtained from the condensation of phenylenediamine with benzaldehyde easily cyclized upon reducing another molecule of imine and was converted into 2-phenylbenzimidazole.[25] Thus, *N*-unsubstituted benzimidazole (7a) was prepared by the condensation of 11 with terephthalaldehydic acid in acidic ethanol. Several *N*-substituted derivatives (7b–e) were prepared directly from 7a by *N*-alkylation.[26]

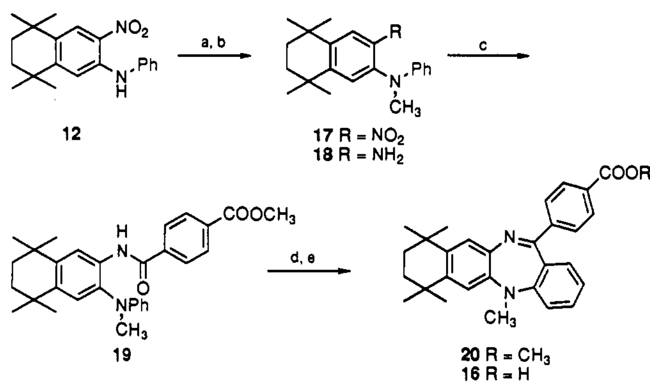
The *N*-phenyl derivative 7f was prepared as shown in Scheme 2. The coupling reaction of the nitroamine 10 with bromobenzene in the presence of copper iodide and

Scheme 2[a]



[a] (a) PhBr/CuI/K_2CO_3; (b) Fe/HCl; (c) p-OHC-Ph-$COOCH_3$/$PhNO_2$/180 °C; (d) NaOH/EtOH (e) p-OHC-Ph-COOH/ethanol/room temperature.

Scheme 3[a]



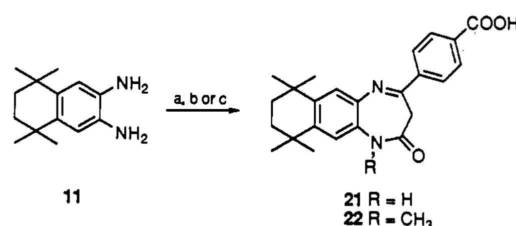
[a] (a) NaH/DMF; CH_3I; (b) Fe/HCl; (c) p-CH_3OOC-Ph-COCl; (d) PPA; (e) NaOH/EtOH.

base,[27] followed by reduction of the nitro group by iron, gave the N-phenyldiamine 13. The condensation of 13 with methyl terephthalaldehydate in nitrobenzene at 180 °C[28] afforded the N-phenylated benzimidazole 14, which was hydrolyzed under basic conditions to give **7f**.

When the condensation of 13 with terephthalaldehydic acid was performed in acidic ethanol at room temperature, **7f** could not be obtained, but a condensed product 15 was obtained. ^{1}H NMR spectroscopic studies showed that 15 has a 1,2-disubstituted benzene moiety as well as 4-substituted benzoic acid and tetramethyltetrahydronaphthalene-2,3-diamine moieties. Furthermore, the signals of one benzylic methine and two secondary amino protons were seen, leading to the structure 15 (Scheme 2). The imine initially formed in the condensation reaction was cyclized by the electrophilic attack to the ortho position of anilino moiety into dibenzodiazepine skeleton.

The formation of the unexpected product 15 in the condensation reaction aroused our interest in the retinoidal activities of compounds with a seven-membered heterocycle as the linking group instead of the five-membered heterocycle in the benzimidazole derivatives. Therefore, three types of compounds with different diazepine rings were designed and synthesized. The synthetic route to 16, an analog of 15 (two secondary amino protons were removed by dehydration), is shown in Scheme 3. The nitroamine 12 was methylated with NaH and methyl iodide, followed by reduction to give the N-methyl-N-phenyldiamine 18. After the condensation of 18 with terephthalic acid monomethyl ester chloride, 19 was

Scheme 4[a]



[a] (a) p-CH_3OOC-Ph-$COCH_2COOC_2H_5$/xylene/140 °C; (b) NaOH/EtOH; (c) NaH/DMF; CH_3I; NaOH/EtOH.

treated with PPA[29] to afford a cyclized compound **20**, which was hydrolyzed to **16**.

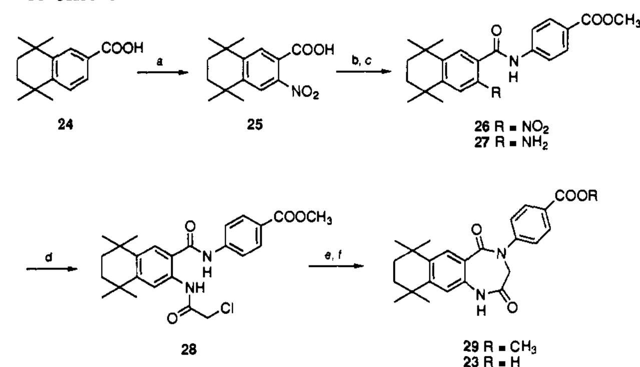
Diazepine derivatives of another type are **21** and its N-methyl derivative **22**, in which the benzo group of **16** is replaced with a methylenecarbonyl group (CH_2CO, Scheme 4). These diazepinone compounds do not have a bulky group on the seven-membered ring and are more hydrophilic than **16**. Compound **21** was prepared by the condensation of the diamine **11** and ethyl [4-(methoxycarbonyl)benzoyl]acetate[30] in xylene at 140 °C,[31] followed by hydrolysis under basic conditions. Compound **22** was prepared by methylation of the condensation product (NaH and methyl iodide) followed by hydrolysis.

A third type of diazepine derivative is **23**, which has another amide bond as the linking group between the two phenyls instead of the imino group of **21**. In our previous studies on the structure–activity relationships of retinobenzoic acids, the introduction of the amide bond as the linking group provided highly potent retinoids such as Am80. Compound **23** was prepared as shown in Scheme 5.[32] Nitration of 5,6,7,8-tetramethyl-5,5,8,8-tetrahydronaphthoic acid (**24**) gave the nitro carboxylic acid (**25**; this nitration of **24** is an interesting process), which was condensed with methyl 4-aminobenzoate, followed by reduction to afford **27**. This product was acylated with chloroacetyl chloride and then heated in methoxyethanol in the presence of base to give **29**, which was hydrolyzed to **23**.

Biological Activity

The ability to induce differentiation of human promyelocytic leukemia cell line HL-60 to mature granulocytes[33,34] was examined as a measure of retinoidal activity. This activity of retinoids correlates well with other retinoidal activities.[1,10] The morphological changes were

Scheme 5[a]



[a] (a) HNO_3/H_2SO_4; (b) $SOCl_2$; p-H_2N-Ph-$COOCH_3$; (c) Fe/NH_4Cl; (d) $ClCH_2COCl$; (e) $K_2CO_3/(C_2H_5)_3N/CH_3OCH_2CH_2OH$; (f) NaOH/EtOH.

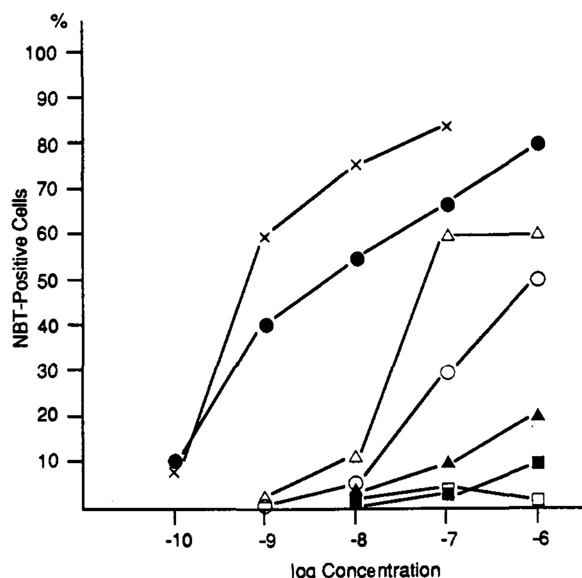


Figure 1. Differentiation-inducing activity of benzimidazole derivatives. Vertical scale is the percentage of differentiated cells evaluated from NBT reduction assay (×) Am80 (**3**), (○) **7a**, (●) **7b**, (△) **7c**, (▲) **7d**, (□) **7e**, (■) **7f**.

examined by a microscopy after Wright–Giemsa staining, and the percentages of the differentiated cells were determined from nitro blue tetrazolium (NBT) reduction assay as a functional marker of differentiation.[35] These two indexes of differentiation correlated well.[36] The activities of the benzimidazole derivatives (**7a–f**) are shown in Figure 1, and the ED_{50} values evaluated from NBT reduction assay are listed in Table 1. The N-unsubstituted benzimidazole derivative **7a** induced the differentiation of HL-60 cells into mature granulocytes at concentrations above 10^{-7} M. Our previous studies on the structure–activity relationships of the retinobenzoic acids indicated that conformational restriction by a five-membered ring, such as a 2-phenylindene structure, an analog of retinoidal stilbenes, sometimes resulted in a decrease of the retinoidal activity.[37] In this case, the conformational restriction of Am80 by the benzimidazole skeleton also decreased the activity. However, the introduction of a methyl group on the N atom of the benzimidazole moiety (**7b**) increased the activity by 2 orders of magnitude, and the ED_{50} of **7b** is 3.4×10^{-9} M. On the other hand, bulkier alkyl substituents on the nitrogen atom adversely affected the retinoidal activity of benzimidazole derivatives: though **7c**, having an N-ethyl group, still showed differentiation-

Table 1. Differentiation-Inducing Activities of Benzimidazole Derivatives

compd	ED_{50},[a] M	rel act.[b]
retinoic acid	2.4×10^{-9}	100
Am80 (**3**)	7.9×10^{-10}	350
7a	3.2×10^{-7}	0.70
7b	3.4×10^{-9}	65
7c	4.6×10^{-8} [c]	4.9
7d	$>10^{-6}$ [d]	$<10^{-2}$
7e	inactive[d] (antagonist)	
7f	inactive[d] (antagonist)	

[a] ED_{50} values of active compounds were calculated from NBT reduction assay data. This is also the case in the other table. [b] The ratio of ED_{50} (retinoic acid) to ED_{50} (a test compound), both values having been obtained in concurrent experiments. This is also the case in the other table. [c] Cellular response to **7c** is maximal at 10^{-7} M (Figure 1). [d] "$>10^{-6}$ M" means there was slight activity at 10^{-6} M. "Inactive" means there was no activity at 10^{-6} M.

Table 2. Differentiation-Inducing Activities of Diazepine Derivatives

compd	ED_{50}, M	rel act.
retinoic acid	2.4×10^{-9}	100
Am80 (**3**)	7.9×10^{-10}	350
15	$>10^{-6}$	$<10^{-2}$
16	inactive (antagonist)	
21	inactive	
22	5.3×10^{-7}	0.43
23	inactive	

inducing activity, its potency was maximal only at 10^{-7} M and its maximum cellular response (60%) is less than that of Am80 (**3**). Introduction of an isopropyl group abolished most of the differentiation-inducing activity toward HL-60 cells, and the addition of **7d** (10^{-6} M) resulted in only 18% of NBT positive cells. Compounds with an even bulkier substituents (**7e** and **7f**) were completely inactive at concentrations below 10^{-6} M.

Differentiation-inducing activities of diazepine derivatives are shown in Table 2. The dibenzodiazepine **15** induced the differentiation of HL-60 cells into mature granulocytes at concentrations above 10^{-6} M, although the responsed cells were less than 20% by 10^{-6} M of **15**. However, **16** was completely inactive at concentrations below 10^{-6} M, although **16** is a more stable analog of **15** in which two secondary amino groups were modified. The replacement of the benzo group of **15** with a methylene-carbonyl group formed the diazepinone **21**, which was also inactive at concentrations below 10^{-6} M. Similarly, the diazepinedione **23** did not exhibit the differentiation-inducing activity. One interpretation of the lack of the activity in these diazepinone derivatives is that the existence of a polar moiety in this area of the seven-membered ring may cause the decrease or disappearance of the activity. Indeed, N-methylation of the amide group of **21** resulted in the significant activity of **22**. A similar effect of the methylation of polar heteroatoms on the diffentiation-inducing activity was also observed in a series of retinoidal quinolinone derivatives (data not shown). From this point of the view, the disappearance of the activity in **16** is a much more interesting result, which led us to examine the antagonistic activity of **16** and other diazepine derivatives as described below.

In order to find retinoid antagonists, the compounds that are inactive on HL-60 differentiation (Figure 2) were examined for their ability to inhibit the differentiation-inducing ability of Am80 (**3**). Benzimidazole derivatives, **7e** and **7f**, significantly inhibited the activity of Am80 at

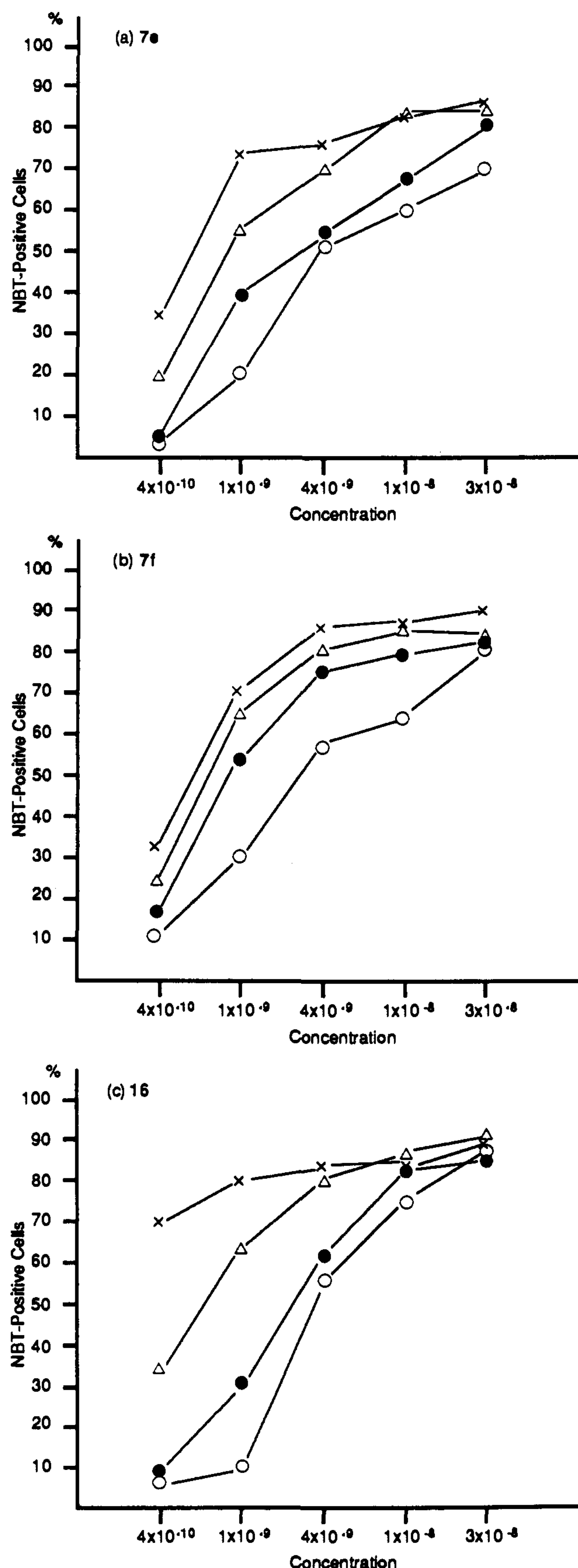


Figure 2. Effect of benzimidazole and diazepine derivatives on HL-60 cell differentiation induced by Am80. Test compounds are (a) **7e**, (b) **7f**, and (c) **16**. In each figure, the vertical scale is differentiated cells evaluated from NBT reduction assay, and the horizontal scale is molar concentration of Am80 (**3**). Concentrations of the added compounds are 1×10^{-6} M (○), 3.3×10^{-7} M (●), 1.1×10^{-7} M (△), and zero (×).

concentrations above 10^{-7} M. The response to Am80 at 4.1×10^{-10} (34% of NBT positive cells) and 1.2×10^{-9} M (75%) was reduced in the presence of 1×10^{-6} M of **7e** to 3% and 20%, respectively, and to 70% even at the concentration of 3.3×10^{-8} M Am80 (86%). The addition of 1×10^{-6}, 3.3×10^{-7}, or 1.1×10^{-7} M **7e** decreased the percentage of the differentiated cells (34%) induced by 4.1×10^{-10} M Am80 to 3%, 3%, or 19%, respectively. Compound **7f**, having an *N*-phenyl group instead of the *N*-benzyl group, also competitively suppressed the activity of Am80, with a similar potency to that of **7e**.

Among the compounds having a seven-membered ring (**16**, **21**, and **23**), only the dibenzodiazepine **16** showed antagonistic activity toward Am80. The potency of **16** is similar to that of **7e** or **7f**. The percentage of differentiated cells (80%) induced by 1.2×10^{-9} M Am80 decreased to 10%, 31%, or 63% in the presence of 1×10^{-6}, 3.3×10^{-7}, or 1.1×10^{-7} M **16**, respectively. Compounds **21** and **23**, though they lack differentiation-inducing activity, did not suppress the Am80-induced differentiation of HL-60 cells (data not shown). These compounds have a hydrophilic amide group in their heterocyclic rings. On the other hand, this region of **16** is occupied by a hydrophobic and bulky group. Thus, **16** can be regarded as having a bulky hydrophobic group (methylanilino group) on the linking group (N=C), so it can be classified into the same category as the antagonistic benzimidazole derivatives **7e** and **7f**, designed from a consideration of the structure–activity relationships of both retinoids and estrogen antagonists (tamoxifen).

The benzimidazole derivatives (**7e** and **7f**) and dibenzodiazepine derivative **16** inhibited the Am80-induced differentiation of HL-60 cells. The responses depended on both the concentrations of Am80 and inhibitors. The maximal responses to Am80 reached the same degree independent of the presence or absence of the inhibitors. Therefore, these compounds inhibited competitively the retinoidal activity of Am80 (**3**). Since HL-60 cells have several subtypes of retinoic acid receptors, RARs,[4] the binding strength of these compounds to some of the receptor types was examined. Although there are many reports on the binding of retinoids to RARs, the binding constants are variable and strongly depend on the source of receptors and the experimental conditions.[9] In this study, the binding abilities of these three compounds were examined by competitive binding experiments with ^{3}H-labeled Am80, as described previously.[4,14] The receptor used was the ligand-binding domain of RAR α that was expressed using pMAL-c2 (NEB) and purified over amylose resin. Its ligand-binding feature is similar to that of hRARα (data not shown).[38] The competition curves for binding to RAR α ligand-binding region are shown in Figure 3. All of the compounds examined bound to RAR α with lower affinity than Am80 (**3**) by 3 orders of magnitude so that the affinity approximately correlates to the ability to suppress the retinoidal activities of Am80 (**3**) on HL-60 cells. The binding strengths were further examined for the selected compounds **7e** and **7f** with RAR α extracted from RAR α-expression vector-transfected COS-1 cells and RAR β extracted from HL-60 cells (Figure 4).[4,39] Both compounds bound to RAR α and β with a similar potency to RAR α ligand-binding domain. In this series of retinoid antagonists, there is no significant difference in the binding abilities to RAR α and β. The binding ability of the synthetic antagonists can be

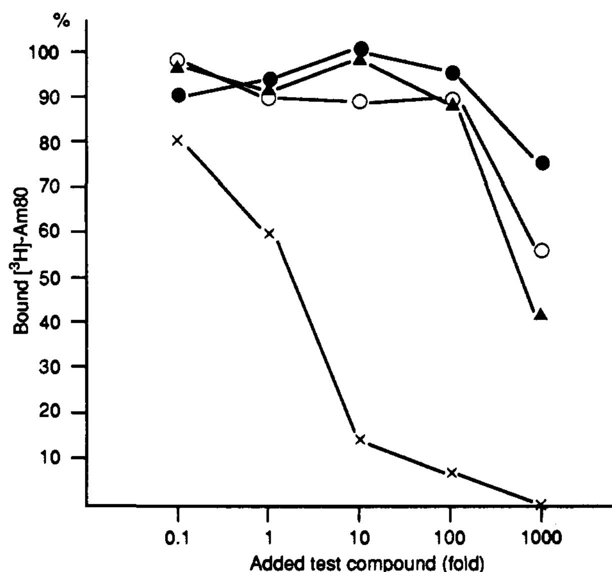


Figure 3. Competitive binding curves to RAR α ligand-binding domain. The vertical scale is percentage of [^{3}H]Am80 bound to RAR α ligand-binding domain. The horizontal scale is the amount (fold units) of the added compounds (×) Am80 (3), (○) 7e, (●) 7f, (▲) 16.

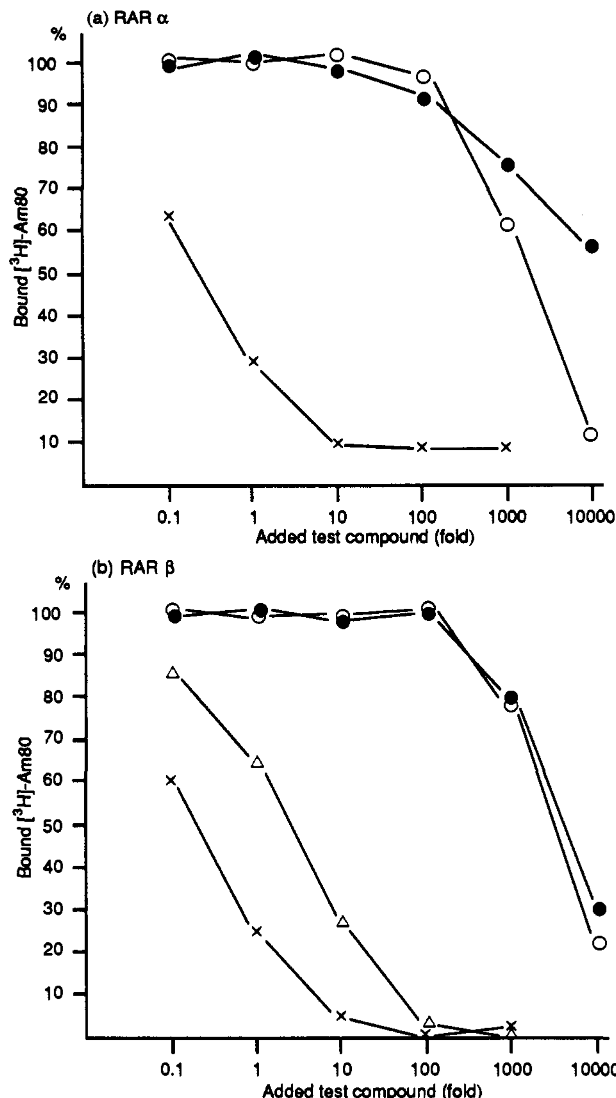


Figure 4. Competitive binding curves to RAR α and β. The vertical scale is percentage of [^{3}H]Am80 bound to RAR α (a) and β (b). The horizontal scale is the amount (fold units) of the added compounds (×) retinoic acid (1), (△) Am80 (3), (○) 7e, (●) 7f.

interpreted in terms of competitive antagonistic activity on the basis of the receptor–ligand interaction.

In conclusion, the benzimidazole derivatives **7e** and **7f** and dibenzodiazepine derivative **16** exhibit antagonistic activity to the potent retinoid Am80 (**3**) by binding to retinoic acid receptors, RARs. These antagonists should be useful tools for the study of retinoidal actions, even though their potencies are weak. They may also help to extend the range of clinical usefulness of retinoids and may themselves have new clinical applications.

Experimental Section

General. Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and were within ±0.3% of the theoretical values. NMR spectra were recorded on JEOL JMN-GX400 (400 MHz) spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane. Mass spectra were recorded on JEOL JMS-DX303 spectrometer. UV spectra were measured in 95% ethanol on a Shimadzu UV200S. IR spectra were taken with a Shimadzu IR-408 infrared spectrometer and expressed in cm^{-1}.

6-Acetamido-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (8). A solution of 2,5-dichloro-2,5-dimethylhexane (4.6 g, 25.5 mmol) in 10 mL of dry CH_2Cl_2 was added dropwise to a suspension of acetanilide (2 g, 15 mmol) and $AlCl_3$ (4 g, 30 mmol) at 0 °C. After 20 min, the mixture was poured into ice water and extracted with CH_2Cl_2. The organic layer was washed with aqueous sodium carbonate and water and dried over $MgSO_4$. After evaporation, the residue was chromatographed on silica gel (eluent: *n*-hexane/AcOEt, 3:1) to give **8** (55%). **8**: colorless prisms; mp 110–112 °C; ^{1}H-NMR ($CDCl_3$) δ 7.34 (d, 1 H, *J* = 8 Hz), 7.29 (dd, 1 H, *J* = 2, 8 Hz), 7.24 (d, 1 H, *J* = 2 Hz), 7.05 (s, 1 H), 2.16 (s, 3 H), 1.67 (s, 4 H), 1.27 (s, 6 H), 1.25 (s, 6 H); IR (KBr) 3150, 1650, 1610 cm^{-1}.

6-Acetamido-7-nitro-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (9). KNO_3 (584 mg, 5.77 mmol) was added to a solution of **8** (1.36 g, 5.5 mmol) in trifluoromethanesulfonic acid (15 mL) at 0 °C, and the mixture was stirred at room temperature for 90 min. The mixture was poured into a large excess of ice water and extracted with AcOEt. The organic layer was washed with water until the aqueous layer showed pH 7 and dried over Na_2SO_4. After evaporation, the crude product was recrystallized from 50% aqueous ethanol to give **9** (52%). **9**: yellow needles (50% aqueous ethanol); mp 121–123 °C; ^{1}H-NMR ($CDCl_3$) δ 10.20 (s, 1 H), 8.72 (s, 1 H), 8.14 (s, 1 H), 2.27 (s, 3 H), 1.70 (s, 4 H), 1.32 (s, 6 H), 1.30 (s, 6 H); IR (KBr) 3350, 1700, 1615 cm^{-1}; MS M$^+$ 290. Anal. ($C_{16}H_{22}N_2O_3$) C, H, N.

5,6,7,8-Tetrahydro-5,5,8,8-tetramethylnaphthalene-2,3-diamine (11). A 25-mL portion of 2 N NaOH was added to a solution of **9** (731 mg, 2.52 mmol) in 30 mL of ethanol, and the mixture was refluxed for 2 h. After cooling, the mixture was extracted with CH_2Cl_2. The organic layer was washed with water, dried over $MgSO_4$, and evaporated to give **10** (99%). **10**: orange oil; ^{1}H-NMR ($CDCl_3$) δ 8.10 (s, 1 H), 6.70 (s, 1 H), 1.67 (s, 4 H), 1.27 (s, 6 H), 1.26 (s, 6 H); IR (KBr) 3450, 1620 cm^{-1}. Next, 450 mg of iron powder was added to a mixture of **10** (270 mg, 1.08 mmol), 0.8 mL of concentrated HCl, and 4 mL of water, and the mixture was heated at 120 °C for 30 min. After basification by sodium carbonate, the mixture was extracted with AcOEt. The organic layer was washed with brine and dried over Na_2SO_4. After evaporation, the crude product was purified by recrystallization to give **11** (76%). **11**: colorless flakes (AcOEt/*n*-hexane); mp 184 °C; ^{1}H-NMR ($CDCl_3$) δ 6.63 (s, 2 H), 3.10 (br s, 4 H), 1.63 (s, 4 H), 1.22 (s, 12 H); IR (KBr) 3300, 1630 cm^{-1}; MS M$^+$ 218.

4-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethylnaphth[2,3-*d*]imidazol-2-yl)benzoic Acid (7a).[25] A 1.4-mL amount of 1 N hydrochloric acid was added to a mixture of terephthalaldehydic acid (210 mg, 1.4 mmol) and **11** (400 mg, 1.4 mmol) in ethanol

(3 mL), and the mixture was stirred for 1 h. The precipitate was collected and dissolved in a small amount of ethanol. **7a** was precipitated by addition of water, collected, and recrystallized from methanol (41%). **7a**: pale yellow prisms (methanol); mp 273–275 °C; ^{1}H-NMR (DMSO-d_6) δ 8.28 (d, 2 H, J = 8.4 Hz), 8.13 (d, 2 H, J = 8.4 Hz), 7.59 (s, 2 H), 1.72 (s, 4 H), 1.34 (s, 12 H); IR (KBr) 1700, 1610 cm^{-1}; UV (95% ethanol) λ_{max} (log ε) 324 nm (4.5). Anal. ($C_{22}H_{24}N_2O_2$·HCl), C, H, N.

4-(5,6,7,8-Tetrahydro-1,5,5,8,8-pentamethylnaphth[2,3-*d*]imidazol-2-yl)benzoic Acid (7b).[26] KOH (90 mg) was added to a suspension of **7a** (90 mg, 0.23 mmol) in 2 mL of acetone. After stirring for 30 min, 0.04 mL of methyl iodide was added to the reaction mixture and was stirred for 24 h. After removal of the solvent, the residue was dissolved in a small amount of water. Next, 2 N hydrochloric acid was added, and the precipitates was collected and recrystallized from methanol to give 7b (93%). **7b**: pale yellow prisms (methanol); mp >300 °C; ^{1}H-NMR (DMSO-d_6) δ 8.23 (d, 2 H, J = 8 Hz), 8.06 (d, 2 H, J = 8 Hz), 8.01 (s, 1 H), 7.74 (s, 1 H), 4.01 (s, 3 H), 1.75 (s, 4 H), 1.40 (s, 6 H), 1.36 (s, 6 H); IR (KBr) 2900, 1710, 1500 cm^{-1}; UV (95% ethanol) λ_{max} (log ε) 310 nm (4.3). Anal. ($C_{23}H_{26}N_2O_2$·HCl) C, H, N.

4-(1-Ethyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphth-[2,3-*d*]imidazol-2-yl)benzoic Acid (7c). **7a** (50 mg, 0.14 mmol) was added to a suspension of NaH (60%; 18 mg, 0.48 mmol) in THF. After 30 min, ethyl iodide (0.01 mL) was added, and the mixture was stirred for 2 weeks. The mixture was poured into ice water. After removal of THF under vacuum, 2 N hydrochloric acid was added dropwise and the precipitates was collected and recrystallized to give **7c** (60%). **7c**: colorless flakes (ethanol/water); mp 290 °C dec; ^{1}H-NMR (DMSO-d_6) δ 8.11 (d, 2 H, J = 8.4 Hz), 7.87 (d, 2 H, J = 8.4 Hz), 7.64 (s, 1 H), 7.55 (s, 1 H), 4.31 (q, 2H, J = 7 Hz), 1.71 (s, 4 H), 1.36 (s, 6 H), 1.33 (s, 6 H), 1.29 (t, 3H, J = 7 Hz); IR (KBr) 1700, 1610 cm^{-1}; UV (95% ethanol) λ_{max} (log ε) 305 nm (4.3). Anal. ($C_{24}H_{28}N_2O_2$·$^1/_4$$H_2O$) C, H, N.

4-(5,6,7,8-Tetrahydro-1-isopropyl-5,5,8,8-tetramethyl-naphth[2,3-*d*]imidazol-2-yl)benzoic Acid (7d). Alkylation of **7a** and isopropyl iodide using NaH as base in THF (2 weeks) by the method described above gave **7d** (16%). **7d**: colorless prisms (methanol); mp 289 °C; ^{1}H-NMR (DMSO-d_6) δ 8.11 (d, 2 H, J = 8.4 Hz), 7.75 (d, 2 H, J = 8.4 Hz), 7.63 (s, 1 H), 7.59 (s, 1 H), 4.65 (m, 1H), 1.71 (s, 4 H), 1.59 (d, 6 H, J = 7 Hz), 1.37 (s, 6 H), 1.32 (s, 6 H). Anal. ($C_{25}H_{30}N_2O_2$·$^3/_4$$H_2O$), C, H, N.

4-(1-Benzyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphth-[2,3-*d*]imidazol-2-yl)benzoic Acid (7e). Alkylation of **7a** and benzyl bromide using NaH as base in THF (room temperature, 2 days) gave by the method described above **7e** (58%). **7e**: colorless prisms (AcOEt/methanol/*n*-hexane); mp 258 °C; ^{1}H-NMR (DMSO-d_6) δ 8.16 (d, 2 H, J = 8.4 Hz), 7.96 (d, 2 H, J = 8.4 Hz), 7.74 (s, 1 H), 7.72 (s, 1 H), 7.30 (m, 3 H), 7.14 (d, 2 H), 5.70 (s, 2 H), 1.70 (s, 4 H), 1.35 (s, 6 H), 1.26 (s, 6 H); UV (95% ethanol) λ_{max} (log ε) 307 nm (4.3); IR (KBr) 3500, 1690, 1610 cm^{-1}; MS M$^+$ 438. Anal. ($C_{29}H_{30}N_2O_2$·$^1/_4$$H_2O$) C, H, N.

(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-3-nitro-2-naphth-yl)phenylamine (12).[23] Potassium carbonate (470 mg, 3.41 mmol) and a small amount of copper iodide were added to a solution of **10** (2.7 g, 10.0 mmol) in 40 mL of bromobenzene, and the mixture was heated at 180 °C for 12 h. After removal of bromobenzene, the residue was dissolved in CH_2Cl_2, washed with water and brine, and dried over $MgSO_4$. The crude mixture was chromatographed on silica gel (eluent: *n*-hexane/AcOEt, 40:1) to give **12** (48%) and the recovery of **10** (48%). **12**: red prisms (ethanol); mp 132 °C; ^{1}H-NMR (CDCl$_3$) δ 9.25 (s, 1 H), 8.14 (s, 1 H), 7.39 (m, 2 H), 7.25 (m, 3 H), 7.18 (m, 1 H), 1.67 (s, 4 H), 1.30 (s, 6 H), 1.18 (s, 6 H); IR (KBr) 1740, 1600 cm^{-1}; MS M$^+$ 324. Anal. ($C_{20}H_{24}N_2O_2$) C, H, N.

***N*-Phenyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene-2,3-diamine (13).** A 0.7-mL amount of concentrated HCl and 330 mg of iron powder was added to a mixture of **12** (250 mg, 0.77 mmol), 4 mL of water, and 2.5 mL of ethanol, and the mixture was refluxed for 80 min. After cooling, the mixture was basified with sodium carbonate and extracted with AcOEt. The organic layer was washed with water and brine and dried over $MgSO_4$. The crude mixture was chromatographed on silica gel (eluent: *n*-hexane/AcOEt, 8:1) to give **13** (40%). **13**: ^{1}H-NMR (CDCl$_3$) δ 7.20 (m, 2 H), 7.07 (s, 1 H), 6.75 (m, 4 H), 5.25 (br s, 1 H), 3.00 (br s, 2 H), 1.65 (s, 4 H), 1.26 (s, 6 H), 1.20 (s, 6 H); IR (KBr) 3400, 3250, 1600 cm^{-1}; MS M$^+$ 294.

4-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-1-phenylnaphth-[2,3-*d*]imidazol-2-yl)benzoic Acid (7f).[28] A mixture of **13** (65 mg, 0.22 mmol) and methyl terephthalaldehydate (36 mg. 0.22 mmol) in nitrobenzene (1 mL) was gradually heated to 180 °C for 1.5 h. After cooling, the reaction mixture was chromatographed on silica gel (eluent: *n*-hexane/AcOEt, 6:1) to give **14** (70%). **14**: colorless flakes (AcOEt/*n*-hexane); mp 241 °C; ^{1}H NMR (CDCl$_3$) δ 7.95 (d, 2 H, J = 8.4 Hz), 7.89 (s, 1 H), 7.60 (d, 2 H, J = 8.4 Hz), 7.52 (m, 3 H), 7.29 (m, 2 H), 7.18 (s, 1 H), 3.90 (s, 3 H), 1.75 (s, 4 H), 1.40 (s, 6 H), 1.29 (s, 6 H); IR (KBr) 1725, 1610 cm^{-1}; MS M$^+$ 438. Anal. ($C_{29}H_{30}N_2O_2$) C, H, N. **14** (32 mg) was hydrolyzed by the usual method using 1 mL of ethanol and 0.4 mL of 2 N NaOH to give **7f**. **7f**: colorless flakes (methanol); mp 297 °C; ^{1}H-NMR (DMSO-d_6) δ 7.88 (d, 2 H, J = 8.4 Hz), 7.77 (s, 1 H), 7.56 (m, 5 H), 7.40 (d, 2 H, J = 7 Hz), 7.09 (s, 1 H), 1.70 (s, 4 H), 1.36 (s, 6 H), 1.23 (s, 6 H); IR (KBr) 1690, 1610 cm^{-1}. Anal. ($C_{28}H_{28}N_2O_2$) C, H, N.

4-(5*H*-7,8,9,10,12,13-Hexahydro-7,7,10,10-tetramethylbenzo[*e*]naphtho[2,3-*b*][1,4]diazepin-13-yl)benzoic Acid (15). Terephthalaldehydic acid (43 mg, 0.28 mmol) was added to a mixture of **13** (84 mg, 0.28 mmol), 0.4 mL of 2 N hydrochloric acid, and 2 mL of ethanol, and the mixture was stirred for 20 h. After evaporation, the residue was dissolved in 2 N NaOH and extracted with AcOEt. The organic layer was washed with water and dried over $MgSO_4$. The crude mixture was purified by silica gel column chromatography (eluent: CH_2Cl_2/CH_3OH, 9:1) to give **15** (67%). **15**: ^{1}H-NMR (DMSO-d_6) δ 7.98 (s, 1 H, NH), 7.76 (d, 2 H, J = 8.1 Hz), 7.14 (d, 2 H, J = 8.1 Hz), 7.05 (t, 1 H, J = 7.5 Hz), 6.91 (d, 1 H, J = 8 Hz), 6.80 (d, 1 H, J = 7 Hz), 6.64 (s, 1 H), 6.58 (s, 1 H), 6.55 (t, 1 H, J = 7.5 Hz), 5.92 (s, 1 H, NH), 5.34 (s, 1 H), 1.51 (s, 4 H), 1.14 (s, 3 H), 1.12 (s, 6 H), 1.06 (s, 3 H); IR (KBr) 3350, 1690, 1600 cm^{-1}; MS M$^+$ 426.

***N*-Methyl-*N*-phenyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene-2,3-diamine (18).** A solution of **12** (630 mg, 1.9 mmol) in 8 mL of dry DMF was added to a suspension of NaH (60%; 84 mg, 2.1 mmol) in 3 mL of dry DMF. After 20 min, 0.24 mL of methyl iodide was added and the mixture was stirred for 20 min. The red solution was poured into ice water and extracted with AcOEt. The organic layer was washed with water and brine and dried over $MgSO_4$. The crude product was recrystallized from ethanol to give **17** (73%). **17**: orange prisms (ethanol); mp 118 °C; ^{1}H-NMR (CDCl$_3$) δ 7.84 (s, 1 H), 7.29 (s, 1 H), 7.18 (t, 2 H, J = 7.3 Hz), 6.78 (t, 1 H, J = 7.3 Hz), 6.64 (d, 2 H, J = 8 Hz), 3.28 (s, 3 H), 1.73 (s, 4 H), 1.33 (s, 6 H), 1.27 (s, 6 H); IR (KBr) 1595, 1520, 1490 cm^{-1}; MS M$^+$ 338. Anal. ($C_{21}H_{26}N_2O_2$) C, H, N. Next, 2.5 mL of concentrated HCl and iron powder (900 mg) were added to a suspension of **17** (756 mg, 2.24 mmol) in water (15 mL) and ethanol (20 mL), and the mixture was heated at reflux for 1 h. After cooling, the mixture was basified with 1 N sodium carbonate, and extracted with AcOEt. The organic layer was washed with water and brine and dried over $MgSO_4$. The crude product was recrystallized from methanol to give **18** (53%). **18**: pale yellow flakes; mp 126 °C; ^{1}H-NMR (CDCl$_3$) δ 7.20 (m, 2 H), 6.96 (s, 1 H), 6.73 (m, 1 H), 6.72 (s, 1 H), 6.64 (m, 2 H), 3.57 (br s, 2 H), 3.21 (s, 3 H), 1.65 (s, 4 H), 1.28 (s, 6 H), 1.18 (s, 6 H); IR (KBr) 3300, 1600, 1500 cm^{-1}. Anal. ($C_{21}H_{28}N_2$) C, H, N.

Methyl 4-[[5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-3-(*N*-methyl-*N*-phenylamino)-2-naphthalenyl]carbamoyl]benzoate (19). Terephthalic monomethyl ester chloride (357 mg, 1.81 mmol) was added to a solution of **18** (504 mg, 1.63 mmol) in 100 mL of dry benzene and 1 mL of pyridine. After 1 h, the solvent was removed and the residue was dissolved in CH_2Cl_2. The organic layer was washed twice with 1 N hydrochloric acid and water and dried over $MgSO_4$. After evaporation, the residue was solidified by adding pentane and recrystallized from ethanol to give **19** (83%). **19**: pale yellow flakes (ethanol); mp 163 °C; ^{1}H-NMR (CDCl$_3$) δ 8.54 (s, 1 H), 8.32 (s, 1 H), 8.00 (d, 2 H, J = 8 Hz), 7.51 (d, 2 H, J = 8 Hz), 7.26 (m, 2 H), 7.12 (s, 1 H), 6.86 (t, 1 H, J = 7.3 Hz), 6.74 (d, 2 H, J = 8 Hz), 3.92 (s, 3 H), 3.29 (s, 3 H), 1.71 (s, 4 H), 1.38 (s, 6 H), 1.23 (s, 6 H); IR (KBr) 3350, 1720, 1675, 1600 cm^{-1}; MS M$^+$ 470. Anal. ($C_{30}H_{34}N_2O_3$) C, H, N.

4-(5*H*-7,8,9,10-Tetrahydro-5,7,7,10,10-pentamethylbenzo[*e*]naphtho[2,3-*b*][1,4]diazepin-13-yl)benzoic Acid (16).[29] A mixture of **19** (615 mg, 1.31 mmol) and polyphosphoric acid (5.8 g) were heated at 140 °C for 30 min. After cooling, the mixture was poured into ice water and extracted with AcOEt. The organic layer was washed with water and brine and dried over $MgSO_4$. The crude product was chromatographed on silica gel (eluent: *n*-hexane/AcOEt, 16:1) to give **20** (66%). **20**: orange prisms (petroleum ether); mp 204–206 °C; ^{1}H-NMR ($CDCl_3$) δ 8.06 (d, 2 H, *J* = 8.4 Hz), 7.83 (d, 2 H, *J* = 8.4 Hz), 7.40 (m, 1 H), 7.22 (s, 1 H), 7.06 (d, 1 H, *J* = 8.1 Hz), 6.96 (m, 2 H), 6.83 (s, 1 H), 3.94 (s, 3 H), 3.26 (s, 3 H), 1.66 (s, 4 H), 1.31 (s, 3 H), 1.27 (s, 3 H), 1.25 (s, 3 H), 1.24 (s, 3 H); IR (KBr) 1725, 1615 cm^{-1}. Anal. ($C_{30}H_{32}N_2O_2$) C, H, N. A 1.5-mL portion of 2 N NaOH was added to a solution of **20** (36 mg, 0.08 mmol) in 2 mL of ethanol, and the mixture was stirred for 2 h. After acidification, the mixture was extracted with CH_2Cl_2. The organic layer was washed with water and dried over $MgSO_4$. The crude product was recrystallized from 50% aqueous ethanol to give **16**. **16**: orange prisms (50% aqueous ethanol); mp 281–285 °C; ^{1}H-NMR ($CDCl_3$) δ 8.13 (d, 2 H, *J* = 8.4 Hz), 7.87 (d, 2 H, *J* = 8.4 Hz), 7.40 (m, 1 H), 7.24 (s, 1 H), 7.07 (d, 1 H, *J* = 8.1 Hz), 6.96 (m, 2 H), 6.84 (s, 1 H), 3.27 (s, 3 H), 1.66 (s, 4 H), 1.32 (s, 3 H), 1.28 (s, 3 H), 1.26 (s, 3 H), 1.25 (s, 3 H); IR (KBr) 1705, 1605 cm^{-1}; MS M$^+$ 438. Anal. ($C_{29}H_{30}N_2O_2$) C, H, N.

4-(1*H*-2,3,7,8,9,10-Hexahydro-7,7,10,10-tetramethyl-2-oxonaphtho[2,3-*b*][1,4]diazepin-4-yl)benzoic Acid (21).[31] Ethyl acetoacetate (846 mg, 6.5 mmol) was added to a solution of sodium (150 mg) in 10 mL of ethanol and was cooled at 0 °C. A solution of terephthalic acid monomethyl ester chloride (1.3 g, 6.5 mmol) in 7 mL of dry ether was gradually added to the mixture. After 1 h, the yellow precipitates was filtered, washed with ether, and dissolved in 5 mL of water. Ammonium chloride (115 mg) and 0.4 mL of 28% ammonia solution were added to the solution. The mixture was kept at 55 °C for 2 h and then extracted with ether. The organic layer was washed with water and dried over $MgSO_4$. The crude was purified by silica gel column chromatography (eluent: *n*-hexane/AcOEt, 8:1) to give ethyl [4-(methoxycarbonyl)benzoyl]acetate as a colorless solid (25%): mp 47–49 °C; ^{1}H-NMR ($CDCl_3$) δ 12.55 (s, 1 H), 8.08 (d, 2 H, *J* = 8.8 Hz), 7.84 (d, 2 H, *J* = 8.8 Hz), 5.73 (s, 1 H), 4.28 (q, 2 H, *J* = 7.5 Hz), 3.95 (s, 3 H), 1.35 (t, 3 H, *J* = 7.15 Hz); IR (KBr) 1715, 1620 cm^{-1}. A solution of ethyl [4-(methoxycarbonyl)benzoyl]acetate (119 mg, 0.48 mmol) in 2 mL of xylene was added dropwise to a solution of **11** (100 mg, 0.46 mmol) in 2 mL of xylene at 140 °C, and the mixture was refluxed for 1.5 h. After cooling, the precipitates were collected and recrystallized from ethanol to give methyl 4-(1*H*-2,3,7,8,9,10-hexahydro-7,7,10,10-tetramethyl-2-oxonaphtho[2,3-*b*][1,4]diazepin-4-yl)benzoate (70%): yellow needles (ethanol); mp 270 °C; ^{1}H-NMR ($CDCl_3$) δ 8.16 (d, 2 H, *J* = 9 Hz), 8.12 (d, 2 H, *J* = 9 Hz), 7.78 (s, 1 H), 7.43 (s, 1 H), 6.94 (s, 1 H), 3.95 (s, 3 H), 3.60 (s, 2 H), 1.72 (s, 4 H), 1.34 (s, 6 H), 1.31 (s, 6 H); IR (KBr) 3100, 1720, 1680, 1610 cm^{-1}; MS M$^+$ 404. Anal. ($C_{25}H_{28}N_2O_3$) C, H, N. Methyl 4-(1*H*-2,3,7,8,9,10-hexahydro-7,7,10,10-tetramethyl-2-oxonaphtho[2,3-*b*][1,4]-diazepin-4-yl)benzoate (57 mg, 0.14 mmol) was hydrolyzed by the usual method in 1 mL of ethanol and 0.7 mL of 2 N NaOH to give **21** (95%). **21**: colorless flakes (methanol); mp 262 °C; ^{1}H-NMR (DMSO-d_6) δ 10.41 (s, 1 H), 8.15 (d, 2 H, *J* = 8.4 Hz), 8.07 (d, 2 H, *J* = 8.4 Hz), 7.35 (s, 1 H), 7.15 (s, 1 H), 3.52 (s, 2 H), 1.68 (s, 4 H), 1.29 (s, 6 H), 1.27 (s, 6 H); IR (KBr) 3200, 1720, 1650, 1610 cm^{-1}. Anal. ($C_{24}H_{26}N_2O_3 \cdot {}^1/_5H_2O$) C, H, N.

4-(1*H*-2,3,7,8,9,10-Hexahydro-1,7,7,10,10-pentamethyl-2-oxonaphtho[2,3-*b*][1,4]diazepin-4-yl)benzoic Acid (22). A suspension of methyl 4-(1*H*-2,3,7,8,9,10-hexahydro-7,7,10,10-tetramethyl-2-oxonaphtho[2,3-*b*][1,4]diazepin-4-yl)benzoate (40 mg, 0.1 mmol) in 1 mL of dry DMF was added to a suspension of NaH (60%; 4.4 mg, 0.11 mmol) in 1 mL of dry DMF. After 30 min, 0.01 mL of methyl iodide was added and the mixture was stirred for 1 h. The mixture was poured into ice water and extracted with AcOEt. The organic layer was washed with water and brine and dried over Na_2SO_4. The crude product was recrystallized from ethanol to give methyl 4-(1*H*-2,3,7,8,9,10-hexahydro-1,7,7,10,10-pentamethyl-2-oxonaphtho[2,3-*b*][1,4]-diazepin-4-yl)benzoate (80%): pale yellow flakes (ethanol); mp 257 °C; ^{1}H-NMR ($CDCl_3$) δ 8.18 (d, 2 H, *J* = 8.8 Hz), 8.12 (d, 2 H, *J* = 8.8 Hz), 7.37 (s, 1 H), 7.20 (s, 1 H), 4.10 (d, 1 H, *J* = 12 Hz), 3.95 (s, 3 H), 3.39 (s, 3 H), 3.10 (d, 1 H, *J* = 12 Hz), 1.73 (s, 4H), 1.39 (s, 3 H), 1.36 (s, 3 H), 1.29 (s, 3 H), 1.25 (s, 3 H); IR (KBr) 1720, 1670, 1610 cm^{-1}. A 0.3-mL amount of 2 N NaOH was added to a suspension of methyl 4-(1*H*-2,3,7,8,9,10-hexahydro-1,7,7,10,10-pentamethyl-2-oxonaphtho[2,3-*b*][1,4]diazepin-4-yl)benzoate (21 mg, 0.05 mmol) in 1 mL of ethanol, and the mixture was stirred overnight. The mixture was acidified and extracted with CH_2Cl_2. The organic layer was washed with water and brine and dried over Na_2SO_4. The crude product was recrystallized from AcOEt to give **22**. **22**: colorless prisms (AcOEt); mp >300 °C; ^{1}H-NMR (DMSO-d_6) δ 8.18 (d, 2 H, *J* = 8 Hz), 8.07 (d, 2 H, *J* = 8 Hz), 7.41 (s, 1 H), 7.33 (s, 1 H), 4.08 (d, 1 H, *J* = 12 Hz), 3.31 (s, 3 H), 3.07 (d, 1 H, *J* = 12 Hz), 1.69 (s, 4 H), 1.35 (s, 6 H), 1.30 (s, 3 H), 1.25 (s, 3 H); IR (KBr) 1715, 1630, 1605 cm^{-1}. Anal. ($C_{25}H_{28}N_2O_3$) C, H, N.

5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-3-nitro-2-naphthoic Acid (25). Fuming nitric acid (2.4 mL) was added dropwise to a solution of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthoic acid (**24**; 5.65 g, 22.4 mmol) in 37.2 mL of sulfuric acid at 0 °C. The mixture was stirred at room temperature for 1 h and was poured into ice water, which was extracted with CH_2Cl_2. The organic layer was washed with water and dried over Na_2SO_4. The crude was recrystallized from AcOEt/*n*-hexane to give **25** (69%). **25**: yellow prisms (AcOEt/*n*-hexane); mp 230 °C; ^{1}H-NMR ($CDCl_3$) δ 7.79 (s, 1 H), 7.78 (s, 1 H), 1.74 (s, 4 H), 1.33 (s, 12 H).

Methyl 4-[(3-Amino-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene-2-carboxamido)benzoate (27). A mixture of **25** (450 mg, 1.6 mmol) and thionyl chloride (3 mL) was heated at 55 °C for 2 h. After removal of excess thionyl chloride, the residue was dissolved in 2 mL of dry ether. A solution of methyl 4-aminobenzoate (245 mg, 1.6 mmol) in 2 mL of dry ether and 0.5 mL of pyridine was added dropwise, and the mixture was stirred overnight. After removal of the solvent, 1 N hydrochloric acid was added to the residue, and the mixture was extracted with AcOEt. The organic layer was washed with water and brine and dried over Na_2SO_4. The crude product was recrystallized from methanol to give **26** (59%). **26**: colorless prisms (methanol); mp 190 °C; ^{1}H-NMR ($CDCl_3$) δ 8.09 (s, 1 H), 8.05 (d, 2 H, *J* = 8.8 Hz), 7.70 (d, 2 H, *J* = 8.8 Hz), 7.59 (s, 1 H), 7.52 (s, 1 H), 3.92 (s, 3 H), 1.74 (s, 4 H), 1.34 (s, 6 H), 1.33 (s, 6 H); IR (KBr) 1700, 1590 cm^{-1}; MS M$^+$ 410. Anal. ($C_{23}H_{28}N_2O$) C, H, N. A 236-mg portion of iron powder was added to a mixture of **26** (300 mg, 0.72 mmol), ammonium chloride (230 mg, 4.3 mmol), methanol (4 mL), and water (4 mL), and the mixture was heated at reflux for 3.5 h. After cooling, the mixture was extracted with ethyl acetate. The organic layer was washed with water and brine and dried over Na_2SO_4. The crude product was recrystallized from ethanol to give **27** (74%). **27**: pale yellow prisms (ethanol); mp 223–224 °C; ^{1}H-NMR ($CDCl_3$) δ 8.05 (d, 2 H, *J* = 8.8 Hz), 8.02 (s, 1 H), 7.67 (d, 2 H, *J* = 8.8 Hz), 7.41 (s, 1 H), 6.67 (s, 1 H), 5.09 (s, 2 H), 3.91 (s, 3 H), 1.67 (s, 4 H), 1.29 (s, 6 H), 1.27 (s, 6 H); IR (KBr) 3550, 3450, 1700, 1660, 1620 cm^{-1}; MS M$^+$ 380. Anal. ($C_{23}H_{28}N_2O_3$), C, H, N.

Methyl 4-[3-(Chloroacetamido)-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene-2-carboxamido]benzoate (28). Chloroacetyl chloride (40 mg, 0.35 mmol) was added to a suspension of **27** (55 mg, 0.15 mmol) in 2 mL of dry ether and 0.1 mL of pyridine at 0 °C, and the mixture was stirred at room temperature for 5 h. The etherous layer was washed with 1 N hydrochloric acid and water, and dried over Na_2SO_4. After the removal of the solvent, the crude was recrystallized from ethanol to give **28** (70%). **28**: colorless flakes (ethanol); mp 274 °C; ^{1}H-NMR ($CDCl_3$) δ 11.16 (s, 1 H), 8.51 (s, 1 H), 8.06 (d, 2 H, *J* = 8.8 Hz), 7.95 (s, 1 H), 7.69 (d, 2 H, *J* = 8.8 Hz), 7.52 (s, 1 H), 4.16 (s, 2 H), 3.93 (s, 3 H), 1.72 (s, 4 H), 1.32 (s, 12 H); IR (KBr) 3250, 1715, 1655, 1595 cm^{-1}; MS M$^+$ 456. Anal. ($C_{25}H_{29}N_2O_4Cl$) C, H, N.

4-(1*H*-2,3,4,5,7,8,9,10-Octahydro-7,7,10,10-tetramethyl-2,5-dioxonaphtho[2,3-*e*][1,4]diazepin-4-yl)benzoic Acid (23).[32] A mixture of **28** (50 mg, 0.11 mmol), 5 drops of methylamine, and potassium carbonate (8 mg, 0.06 mmol) in 1 mL of methoxyethanol was heated at 80 °C for 2.5 h. After cooling, the mixture was diluted with AcOEt, washed with water and brine, and dried over Na_2SO_4. After removal of the solvent, the crude product was recrystallized from methanol to give **29** (89%). **29**: colorless flakes (methanol); mp 294 °C; ^{1}H-NMR ($CDCl_3$) δ 8.10 (d, 2 H,

J = 8.8 Hz), 7.97 (s, 1 H), 7.78 (s, 1 H), 7.62 (d, 2 H, J = 8.8 Hz), 6.89 (s, 1 H), 4.33 (s, 2 H), 3.93 (s, 3 H), 1.72 (s, 4 H), 1.33 (s, 6 H), 1.30 (s, 6 H); IR (KBr) 3200, 1720, 1690, 1650, 1605 cm^{-1}. **29** (39 mg) was hydrolyzed by the usual method in 1 mL of ethanol and 0.4 mL of 2 N NaOH to give **23**. **23**: colorless flakes (AcOEt/n-hexane); mp >300 °C; ^{1}H-NMR (DMSO-d_6) δ 10.49 (s, 1 H), 8.00 (d, 2 H, J = 8.4 Hz), 7.78 (s, 1 H), 7.57 (d, 2 H, J = 8.4 Hz), 7.13 (s, 1 H), 4.26 (s, 2 H), 1.68 (s, 4 H), 1.27 (s, 12 H); IR (KBr) 3150, 1715, 1650, 1600 cm^{-1}. Anal. ($C_{24}H_{26}N_2O_4 \cdot {}^1/_2H_2O$) C, H, N.

Cells and Culture. The human promyelocytic leukemia cell line HL-60[33] was provided by Prof. F. Takaku (Faculty of Medicine, University of Tokyo) and was maintained in continuous suspension culture. The cells were cultured in plastic flakes in RPMI1640 medium, supplemented with 5% fetal bovine serum (FCS) and antibiotics (penicillin G and streptomycin), at 37 °C in a humidified atmosphere at 5% CO_2 in air.

Differentiation-Inducing Assay. Test compounds were dissolved in ethanol at 2 mM and added to the cells, which were seeded at about 8×10^4 cells/mL; the final ethanol concentration was kept below 0.5%. Control cells were given only the same volume of ethanol. Retinoic acid or Am80 (**3**), as a positive control, was always assayed at the same time. The cells were incubated for 4 days and stained with Wright–Giemsa in order to find the morphological change. The percentages of the differentiated cells were determined by nitro-blue tetrazolium (NBT) reduction assay as described.[35] Cells were incubated for 20 min at 37 °C in RPMI1640 medium (5% FCS) and an equal volume of phosphate-buffered saline (PBS) containing NBT (0.2%) and 12-O-tetradecanoylphorbol 13-acetate (TPA; 200 ng/mL). The percentage of cells containing blue-black formazan was determined on a minimum of 200 cells. The evaluation of the differentiation from NBT reduction assay always agreed with the morphological result.[36]

The assays of test compounds were performed at least twice. ED_{50} values of active compounds were calculated from the NBT reduction assay data by means of the van der Waerden method. Relative activities were calculated as the ratio of the ED_{50} of retinoic acid to the ED_{50} of the test compound obtained in concurrent experiments, multiplied by 100. The inhibition of the differentiation-inducing activity of Am80 (**3**) was examined in the absence or presence of the proper concentration of the test compound according to the method described above. In this experiment, the independent effects of Am80 (**3**) and the test compound were always assayed, and the percentages of differentiated cells were determined morphologically and functionally by NBT reduction assay described above.

Materials for Binding Assay. [^{3}H]Am80 (65 Ci/mmol) was prepared at Amersham. Plasmid-containing human RAR cDNA, pSG5-RARα0, was kindly provided by Prof. P. Chambon (INSERM, Strasbourg, France). Oligonucleotides were synthesized with an Applied Biosynthesis Model 391 DNA synthesizer and purified by reversed-phase HPLC. Protein fusion and purification system, including prokaryotic expression vector pMAL-c2, is commercially available (New England Biolabs).

Preparation of Recombinant Ligand-Binding Region of RARα.[38] Prokaryotic expression vector pMAL-c2 was used for the expression of the ligand-binding domain of RARα. Insertion of a gene into pMAL-c2 creates a gene fusion with the MBP (maltose-binding protein)-encoding *mal* E gene, which results in the expression of an MBP-fusion protein. pSG5-RARα0 was digested with SacI and BamHI. The SacI-BamHI fragment corresponding to the E and F domains of RARα was ligated into the XmnI and BamHI sites of pMAL-c2 by including synthetic SacI adaptor in the ligation mixture to give pMAL-c2-RARα(E). The *Escherichia coli* strain JM109 transformed with this plasmid was precultured in LB medium with ampicillin (50 μg/mL) at 37 °C overnight. This culture was diluted 1:100 into M9 medium supplemented with 0.2% casamino acids, 0.5% glucose, and ampicillin and was grown at 30 °C. After 3–4 h (A_{600} = 0.8–1.0), IPTG was added to 0.3 mM to induce expression. After an additional 2–3 h, cells were harvested by centrifugation. The cells were treated with lysozyme and resuspended in extraction buffer (50 mM Tris-HCl, pH 8.0, 4 °C, 100 mM NaCl, 10% glycerol, 10 mM 2-ME, 1 mM EDTA, 1 mM PMSF) and lysed by sonication on ice. DNaseI (10 units/mL) and 10 mM $MgCl_2$ were added to digest DNA, and the mixture was incubated on ice for 30 min. The cell debris was removed by centrifugation at 10000g, 4 °C, for 1 h. Expressed recombinant fusion protein MBP-RARα/E, which contains from Val 184 to Pro 462 of RARα, with affinity-purified with amylose column according to manufacture's protocol. The purified protein was used for retinoid-binding assay or stored at −80 °C.

Preparation of Cell Extract from Cultured Cell Line.[39] Culture and transfection of COS-1 cells with pSG5-RARα0 were performed as described previously.[4b] Pelleted cells, fresh or frozen in liquid nitrogen, were resuspended in 3–10 volumes of cold buffer:0.6 M KCl, 20 mM Tris-HCl, pH 8.0, 1.5 mM EDTA, 1 mM PMSF, 1 μg/mL pepstatin, 0.1 mg/mL bacitracin, 0.1 mM leupeptin, 0.1 mg/mL aprotinin). The suspension was homogenized with a teflon-glass homogenizer and centrifuged at 100000g, 4 °C. The supernatant was used in retinoid-binding assay.

The extract from HL-60 cells was samely prepared as described above, except that the concentration of KCl was 0.3 mM, and was used as crude extract of RARβ.

Retinoid-Binding Assay. The retinoid-binding activity of RARs was estimated by the nitrocellulose filter binding assay method as described previously.[4,14] In case of recombinant MBP-RARα/E, affinity-purified protein (2–3 μg/assay) was diluted with a binding assay buffer (20 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 1 mM EDTA, 10 mM 2ME, 0.2 mM PMSF) and incubated with 4 nM [^{3}H]Am80 in the presence or absence of an unlabeled competitor at 4 °C for 10–16 h. The incubation mixture was absorbed by suction onto a nitrocellulose membrane. The membrane was washed three times with washing buffer (20 mM Tris-HCl, pH 8.0, 0.15 M NaCl) and then with 25% ethanol in distilled water. Radioactivity that remained on the membrane was measured in Atomlight (NEN) by using a liquid scintillation counter.

Abbreviations: PMSF, phenylmethanesulfonyl fluoride; IPTG, isopropyl thio-β-D-galactoside; 2ME, 2-mercaptoethanol.

Acknowledgment. The authors are grateful to Prof. P. Chambon (INSERM, France) for his generous supply of RAR-expression vector.

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