Regulation of Retinoidal Actions by Diazepinylbenzoic Acids.[1] Retinoid Synergists Which Activate the RXR−RAR Heterodimers

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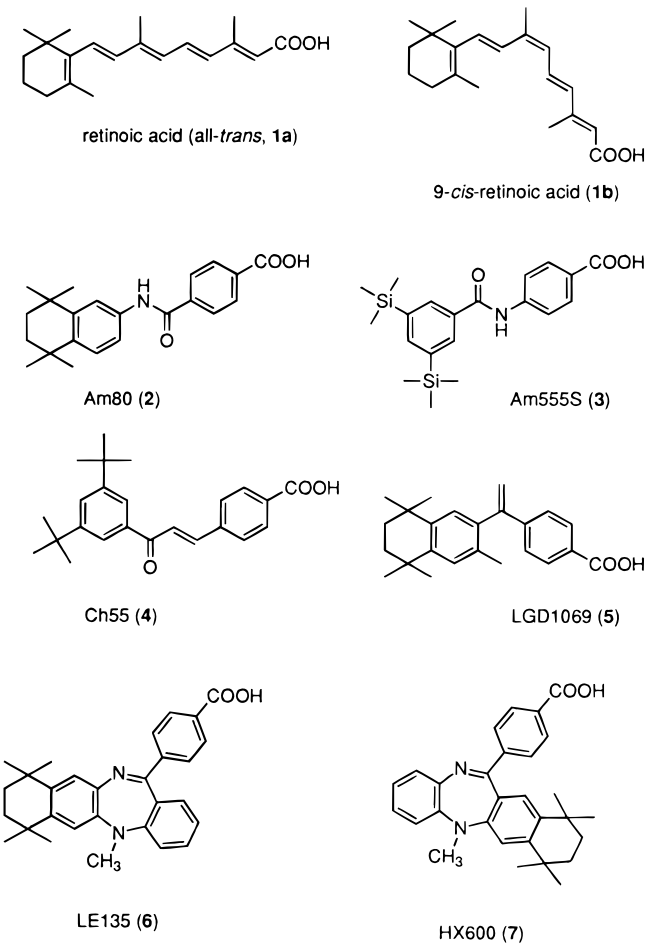
Received June 30, 1997[⊗]

In human HL-60 promyelocytic leukemia cells, diazepinylbenzoic acid derivatives can exhibit either antagonistic or synergistic effects on the differentiation-inducing activities of natural or synthetic retinoids, the activity depending largely on the nature of the substituents on the diazepine ring. Thus, a benzolog of the retinoid antagonist LE135 (**6**), 4-(13*H*-10,11,12,13-tetrahydro-10,10,13,13,15-pentamethyldinaphtho[2,3-*b*][1,2-*e*]diazepin-7-yl)benzoic acid (LE540, **17**), exhibits a 1 order of magnitude higher antagonistic potential than the parental LE135 (**6**). In contrast, 4-[5*H*-2,3-(2,5-dimethyl-2,5-hexano)-5-methyldibenzo[*b*,*e*][1,4]diazepin-11-yl]-benzoic acid (HX600, **7**), a structural isomer of the antagonistic LE135 (**6**), enhanced HL-60 cell differentiation induced by RAR agonists, such as Am80 (**2**). This synergistic effect was further increased for a thiazepine, HX630 (**29**), and an azepine derivative, HX640 (**30**); both synergized with Am80 (**2**) more potently than HX600 (**7**). Notably, the negative and positive effects of the azepine derivatives on retinoidal actions can be related to their RAR-antagonistic and RXR-agonistic properties, respectively, in the context of the RAR−RXR heterodimer.

Retinoids, i.e., retinoic acid (*all-trans*, **1a**, Chart 1) and its analogues, modulate various biological functions, such as cell differentiation, proliferation, and embryonic development in vertebrates.[2] Retinoids also have potential chemopreventive and therapeutic applications in the fields of dermatology and oncology.[3] Retinoic acid (**1a**) has a remarkable remedial effect on acute promyelocytic leukemia (APL).[4] Further, the inhibitory effect of retinoids on IL-6 production suggests their possible usefulness in various IL-6-associated diseases, including psoriasis and rheumatoid arthritis.[5] Some synthetic retinoids have been successfully used in the treatment of psoriasis.[6] However, retinoid therapy is still restricted by the wide range of undesirable side effects. Therefore, it is important to understand the mechanistic basis of the pleiotropic retinoidal activities to provide the basis for the design of action-specific retinoids with extended clinical applicability.

It is well established that retinoidal activities result mainly from the transcriptional regulation of specific gene programs. Retinoids bind to and transactivate two classes of receptors, retinoic acid receptors (RARα, -*β*, -*γ*) and retinoid X receptors (RXRα, -*β*, -*γ*), both of which belong to the steroid/thyroid nuclear receptor superfamily.[2,7] RARs bind retinoic acid (**1a**) and its 9-*cis*-isomer (**1b**), while RXRs bind only 9-*cis*-retinoic acid (**1b**). It is generally accepted that the biological activities of retinoids are mediated by RAR−RXR heterodimers whose subunits follow a defined hierarchy of ligand responsiveness. Recent results have demonstrated that, although both the RAR and RXR subunits can efficiently bind their cognate ligands, only the RAR partner can autonomously respond to its cognate ligands. In contrast, RXR ligands can contribute to the transcriptional activity of the heterodimer only when the

Chart 1



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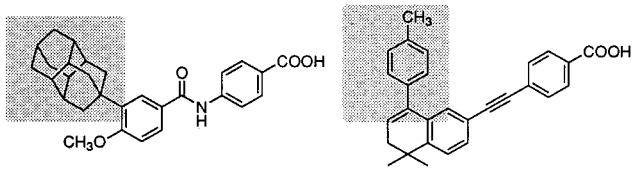
[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1997.

S0022-2623(97)00430-5 CCC: $14.00

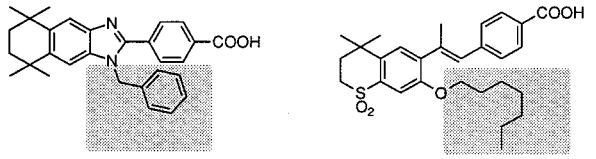
Chart 2

Type I Antagonists

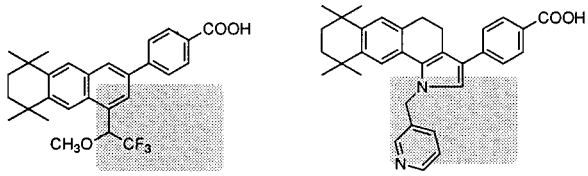


TD550 (**8**) AGN193109 (**9**)

Type II Antagonists



BIBn (**10**) Ro41-5253 (**11**)



SR11335 (**12**) (**13**)

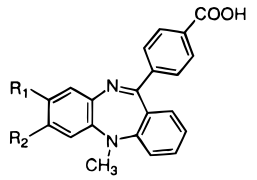
RAR partner is liganded, thus giving rise to the concept of RXR subordination.[8]

Importantly, recent efforts in the design of synthetic retinoids and the availability of 3D structure information[9] have led to the identification of retinoids with improved specificity and/or functionality. Various compounds showing selective affinities for the receptors, i.e., RAR-, RXR-, or subtype-selective retinoids, have been reported.[10−12] For example, Am80 (**2**) and Am555S (**3**) are retinoids which lack RARγ-activation potential[10] but nevertheless exhibit most of the retinoidal activities, like retinoic acid (**1a**).[13] Ch55 (**4**) is an extraordinarily potent synthetic RAR pan-agonist which does not bind to CRABP (cellular retinoic acid-binding protein). In support of these efforts, distinct roles of RAR- and RXR-selective ligands in cell proliferation, differentiation, and apoptosis were recently reported.[14]

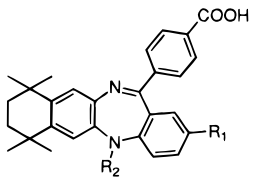
In contrast to the structural variety of retinoid agonists,[2] only a few compounds are presently known to display antagonistic activity; some of them exhibit subtype-selectivity or affinities to RARs as high as those of retinoid agonists.[15−17] In structural terms most of the retinoid antagonists consist of a bulky hydrophobic group on an agonist skeleton and may be classified into two types; type I antagonists (Chart 2) have a large group in the hydrophobic region [for example, the 2,5-dimethyl-2,5-hexano group of Am80 (**2**)] of retinoid agonists, while the type II antagonists have a bulky hydrophobic group in the middle of the molecule, around the linking group between the alkylated aromatic and the benzoic acid moieties.

Previously, we reported the characterization of two isomeric dibenzodiazepine derivatives, LE135 (**6**) and HX600 (**7**), which exert opposite regulatory activities when combined with "classical" retinoid agonists (Chart 1). LE135 (**6**) can bind selectively to RARα and RARβ, with higher affinity to RARβ, and inhibits the retinoic acid (**1a**) or Am80 (**2**)-induced differentiation of human promyelocytic leukemia cells HL-60.[15c,18] On the other hand, HX600 (**7**) enhances the differentiation-inducing activities of retinoic acid (**1a**) or Am80 (**2**) in HL-60 assay.[19] Mechanistic investigation showed that HX600 (**7**) binds to RXR of RXR−RAR heterodimers to synergize with RAR ligands.[20] This positive regulation of retinoidal actions by HX600 (**7**), which is completely inactive alone, is a specific nuclear phenomenon, and should be distinguished from the combination of retinoids with interferons, dibutyryl cAMP, and so on.[21] Recently, RXR-selective ligands have been reported to exhibit synergism with retinoid agonists,[22] but the activity of HX600 (**7**) is characteristically different from that of RXR ligands such as LGD1069 (**5**).[20] In this paper, we describe the design, synthesis, and retinoid-regulatory activities of various dibenzodiazepines and related compounds.

Chart 3

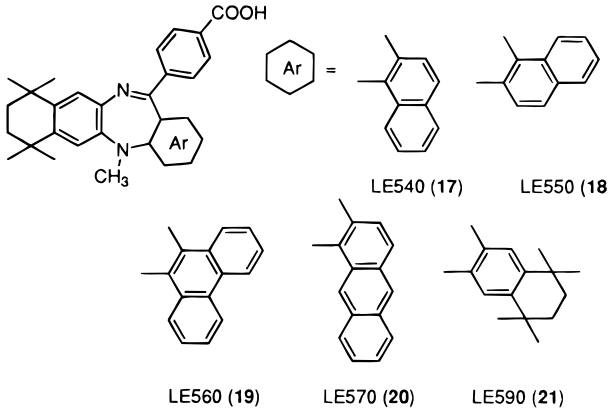


LE510 (**14a**) R_1 = *iso*Pr, R_2 = *iso*Pr
LE511 (**14b**) R_1 = *tert*Bu, R_2 = H
LE515 (**14c**) R_1 = 1-Adamantyl, R_2 = H



LE523 (**15a**) R_1 = H, R_2 = *n*-Pr
LE527 (**15b**) R_1 = H, R_2 = *n*-heptyl
LE531 (**16**) R_1 = NO_2, R_2 = H

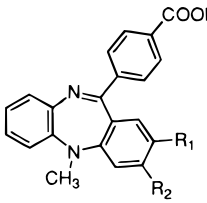
Chart 4



LE540 (**17**) LE550 (**18**)

LE560 (**19**) LE570 (**20**) LE590 (**21**)

Chart 5

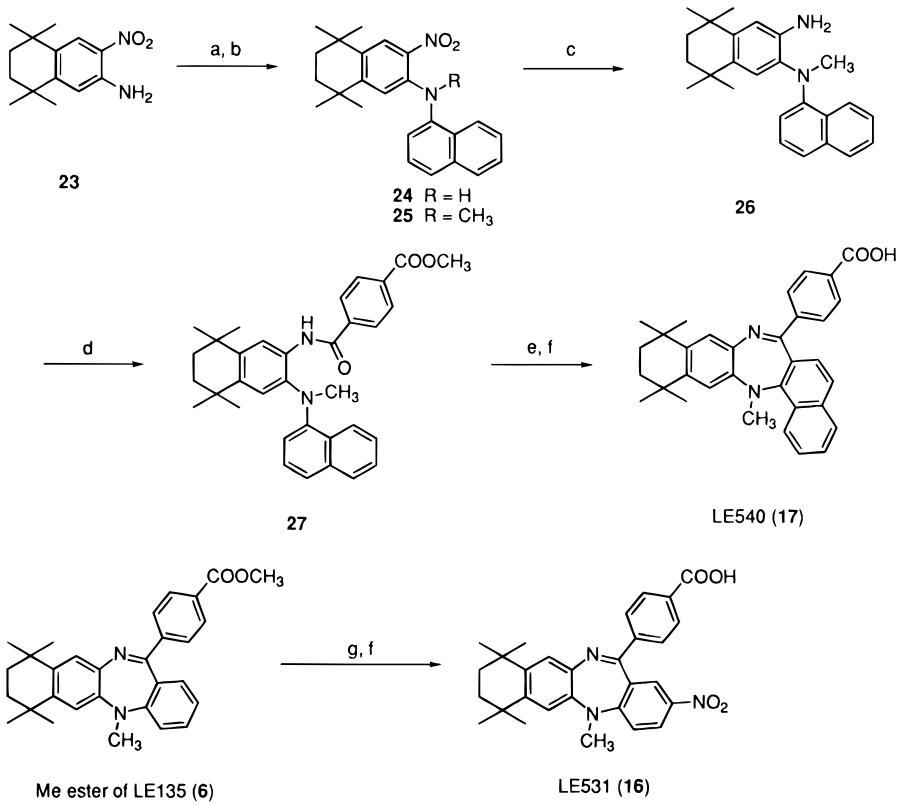


HX500 (**22a**) R_1 = H, R_2 = H
HX511 (**22b**) R_1 = *tert*Bu, R_2 = H
HX513 (**22c**) R_1 = Br, R_2 = H
HX514 (**22d**) R_1 = Ph, R_2 = H
HX515 (**22e**) R_1 = 1-adamantyl, R_2 = H
HX610 (**22f**) R_1 = *iso*Pr, R_2 = *iso*Pr

Results

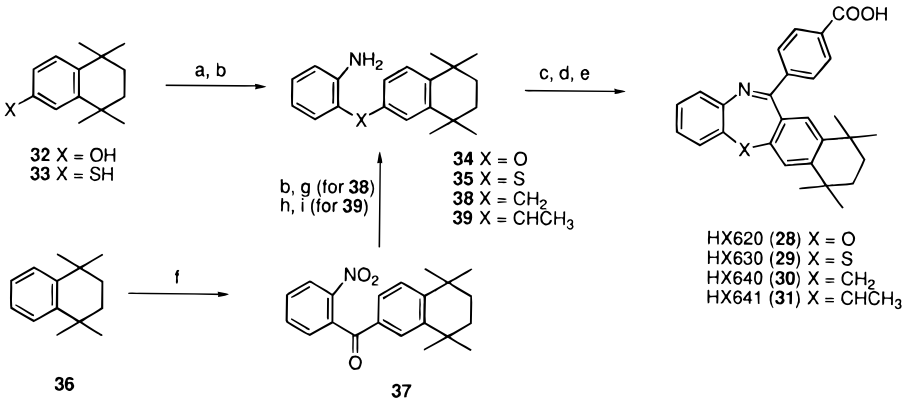
Chemistry. Various diaryldiazepinylbenzoic acids (**14**−**22**) shown in Charts 3−5 were synthesized similarly to LE135 (**6**).[15c] As an example, the synthetic method for LE540 (**17**) is illustrated in Scheme 1. Reaction of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-3-nitro-2-naphthylamine (**23**) with 1-bromonaphthalene in the presence of copper iodide, followed by methylation with NaH and methyl iodide, gave the tertiary amine **25**. When **25** was reduced with Fe under acidic conditions, significant migration of the *N*-naphthyl group also

Scheme 1[a]



[a] (a) 1-Bromonaphthalene/copper iodide/K_2CO_3/Δ; (b) NaH; CH_3I; (c) H_2/Pd−C; (d) *p*-$CH_3OOCPhCOCl$; (e) Δ/polyphosphoric acid; (f) NaOH/EtOH; (g) KNO_3/H_2SO_4.

Scheme 2[a]



[a] (a) 1-Chloro-2-nitrobenzene/KOH/DMSO; (b) Fe/HCl; (c) *p*-$CH_3OOCPhCOCl$; (d) Δ/polyphosphoric acid; (e) NaOH/EtOH; (f) $AlCl_3$/2-nitrobenzoyl chloride; (g) $LiAlH_4$; (h) Ph_3PCH_3Br/*n*-BuLi; (i) H_2/Pd−C.

occurred to afford a mixture of the desired *N*-methyl-*N*-(1-naphthyl)diamine **26** and the isomeric *N*-methyl-*N′*-(1-naphthyl)diamine. Similar migration did not occur when the *N*-aryl group was a 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl or 2-naphthyl group, as in the intermediates for the synthesis of HX600 (**7**) or LE550 (**18**), respectively. Compound **25** was successfully reduced by catalytic hydrogenation to give **26** (86%), which was acylated with terephthalic acid monomethyl ester chloride to give the amide **27** in 95%. Treatment of **27** with polyphosphoric acid, followed by basic hydrolysis of the methyl ester, afforded LE540 (**17**). Introduction of a nitro group on the unsubstituted benzo group was performed by nitration of the methyl ester of LE135 (**6**) using KNO_3 in sulfuric acid, to afford LE531 (**16**, Scheme 1).

The oxa-, thia-, and monoazepine derivatives (**28**−**31**) of HX600 (**7**) were prepared according to the method shown in Scheme 2. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthol (**32**) or the corresponding thiol (**33**) was reacted with 1-chloro-2-nitrobenzene in the presence of KOH in DMSO, followed by reduction of the nitro group with Fe under acidic conditions, to give the diphenyl ether (**34**) or thioether derivative (**35**), respectively. Friedel−Craft acylation of 1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (**36**) with 2-nitrobenzoyl chloride gave **37**. Two-step reduction of the nitro group with Fe/HCl/EtOH and then the keto group with $LiAlH_4$ gave a diphenylmethane derivative **38**. Wittig reaction of **37** with methyltriphenylphosphonium bromide, followed by catalytic hydrogenation, afforded a α-methyldiphenylmethane derivative, **39**. Acylation of **34**, **35**, **38**, and **39** with terephthalic acid monomethyl ester chloride, followed by cyclization and ester hydrolysis,

Table 1. Antagonistic Activity of Diaryldiazepine Derivatives on Am80 (**2**)-Induced HL-60 Cell Differentiation

compound	IC_{50}, M[a]	compound	IC_{50}, M[a]
LE135 (**6**)	1.5×10^{-7}	LE531 (**16**)	2.8×10^{-7}
LE510 (**14a**)	inactive[b]	LE540 (**17**)	3.6×10^{-8}
LE511 (**14b**)	$> 1.0 \times 10^{-6}$ [b]	LE550 (**18**)	1.2×10^{-7}
LE515 (**14c**)	inactive	LE560 (**19**)	3.5×10^{-7}
LE523 (**15a**)	1.3×10^{-7}	LE570 (**20**)	3.2×10^{-7}
LE527 (**15b**)	2.1×10^{-7}	LE590 (**21**)	8.3×10^{-7}

[a] IC_{50} was determined as the concentration of a test compound which reduces by half the percentage of differentiated HL-60 cells induced by 1×10^{-9} M Am80 (**2**). [b] "Inactive" means there was no activity at 1.0×10^{-6} M test compound. "$> 1.0 \times 10^{-6}$ M" means there was slight inhibitory activity at 1.0×10^{-6} M test compound.

gave HX620 (**28**), HX630 (**29**), HX640 (**30**), and HX641 (**31**), respectively.

The 2-oxodiazepines HX800 (**40**) and HX801 (**41**) are derivatives lacking one benzo group of HX600 (**7**). Friedel−Craft acylation of 1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (**36**) with terephthalic acid monomethyl ester chloride gave **42**. Nitration of **42** with KNO_3 in sulfuric acid afforded a single nitrated compound **43** in 53% yield. After reduction of the nitro group, **44** was treated with glycine methyl ester hydrochloride in pyridine to give a 2-oxodiazepine **45** in 33% yield. HX801 (**41**) was obtained by *N*-methylation of **45** with NaH and methyl iodide, followed by hydrolysis of the ester.

Retinoid-Antagonistic Activities (LE Series). The activities of diazepine derivatives were evaluated in terms of the ability to induce or to affect retinoid-induced differentiation of human promyelocytic leukemia cells HL-60.[23,24] The morphological changes were examined by microscopy after Wright−Giemsa staining, and the differentiation state was determined by means of nitro blue tetrazolium (NBT) reduction assay as a functional marker of differentiation.[25] These two indexes of differentiation correlate well with each other.[26] We initially evaluated the antagonistic activity of LE135-related compounds functionally as the inhibitory potency on the differentiation-inducing activity of Am80 in HL-60 cells.

All the azepine derivatives (**14**−**22**) were completely inactive by themselves below 1×10^{-6} M in HL-60 assay. The IC_{50} values of the diazepine derivatives (LE series) on 1×10^{-9} M Am80 (**2**)-induced HL-60 cell differentiation are shown in Table 1. As reported previously, LE135 (**6**) antagonized the differentiation-inducing activity of Am80 (**2**),[15c] and the IC_{50} value is 1.5×10^{-7} for 1.0×10^{-9} M Am80 (**2**). Replacement of the cyclic alkyl group of LE135 (**6**) with bulky acyclic alkyl group(s) diminished the antagonistic activity, as seen for **14a**−**c**. The 2,5-dimethyl-2,5-hexano group of LE135 (**6**) is important for the activity, as in the case of aromatic retinoid agonists.[13] Two diazepines with a longer *N*-alkyl group, LE523 (**15a**) and LE527 (**15b**), were as active as LE135 (**6**), which has an *N*-methyl group.

The hydrophobic benzo group (unsubstituted) of LE135 (**6**) seems to be essential for its antagonistic activity.[15c] Therefore, several compounds with modifications of the benzo group were synthesized, and their retinoid-antagonistic activities were examined. Introduction of a nitro group onto LE135 (**6**), yielding LE531 (**16**), slightly decreased the activity (Table 1). Increase of the antagonistic activity was observed when the benzo group of LE135 (**6**) was replaced with a naphtho group. Thus, LE540 (**17**) is a more potent retinoid antagonist. HL-60 cell differentiation induced by 3.0×10^{-9} M Am80 (**2**) was completely inhibited by addition of 3.0×10^{-7} M LE540 (**17**) and was reduced by half by 1.0×10^{-7} M LE540 (**17**). LE550 (**18**), an isomer of LE540 (**17**), is as active as LE135 (**6**). Introduction of a bulkier aromatic moiety such as a phenanthrene or anthracene ring instead of the naphtho group seems to be ineffective, since both LE560 (**19**) and LE570 (**20**) were rather weaker antagonists than LE135 (**6**). LE590 (**21**), possessing two 5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphtho groups, also exhibited antagonistic activity in HL-60 assay (Figure 1b). Interestingly, 1.0×10^{-6} M LE590 (**21**) decreased the potency of Am80 (**2**) only by up to half, regardless of the concentration of Am80 (**2**), which indicated a noncompetitive antagonistic character of LE590 (**21**).

Structural Evolution from Retinoid Antagonists to Retinoid Synergists (HX Series). A cyclic alkyl moiety (2,5-dimethyl-2,5-hexano group) is necessary in the dibenzodiazepine structure of the retinoid antagonist LE135 (**6**), possibly in order to bind to and antagonize RARs. However, the introduction of another 2,5-dimethyl-2,5-hexano group on the unsubstituted benzo group of LE135 (**6**) generated the noncompetitive antagonist LE590 (**21**), although the hydrophobic aromatic region is important, as seen in LE540 (**17**). The two tetrahydrotetramethylnaphtho moieties of LE590 (**21**) seem to have different roles in eliciting its biological activity. This hypothesis led us to synthesize HX600 (**7**), an isomer of LE135 (**6**), with the cyclic alkyl group on a different aromatic ring, i.e., structurally, HX600 (**7**) may lack the necessary hydrophobic group at the proper position for binding to RARs.

HX600 (**7**) itself showed absolutely no retinoidal activity in the HL-60 assay, but dose-dependently enhanced the differentiation-inducing activities of Am80 (**2**) as shown in Figure 2a.[19] For example, the percentage of 1.0×10^{-9} M Am80 (**2**)-induced differentiated cells (44%) was increased to 65% and 90% by addition of 1.0×10^{-8} and 1.0×10^{-7} M HX600 (**7**), respectively.

Various azepine derivatives related to HX600 (**7**) were examined for retinoid synergistic activities based on HL-60 cell differentiation. The synergistic effective concentration (SEC_{50}) value shown in Table 2 was defined as the EC_{50} of the synergist in combination with 3.0×10^{-10} M Am80 (**2**), which alone induces HL-60 cell differentiation to the extent of ca. 20%. HX500 (**22a**) with no alkyl group on the benzo groups was inactive below 1.0×10^{-6} M. In contrast to the diminution of the antagonistic activity of LE510 (**14a**), HX610 (**22f**) with two acyclic alkyl groups exhibited synergism with Am80 (**2**), and its SEC_{50} value was 6.3×10^{-9} M in the presence of 3.0×10^{-10} M Am80 (**2**). Among compounds having one substituent (R_1 in Chart 3), only HX515 (**22e**) exhibited synergistic activity with Am80 (**2**), although its potency is weak.

HX620 (**28**), the oxazepine analogue of HX600 (**7**), showed similar retinoid synergistic activity with Am80 (**2**). Improvement of the synergistic activity was observed in HX630 (**29**) and HX640 (**30**), the thiazepine and the monoazepine analogues of HX600 (**7**), respectively. The activities of HX630 (**29**) and HX640 (**30**)

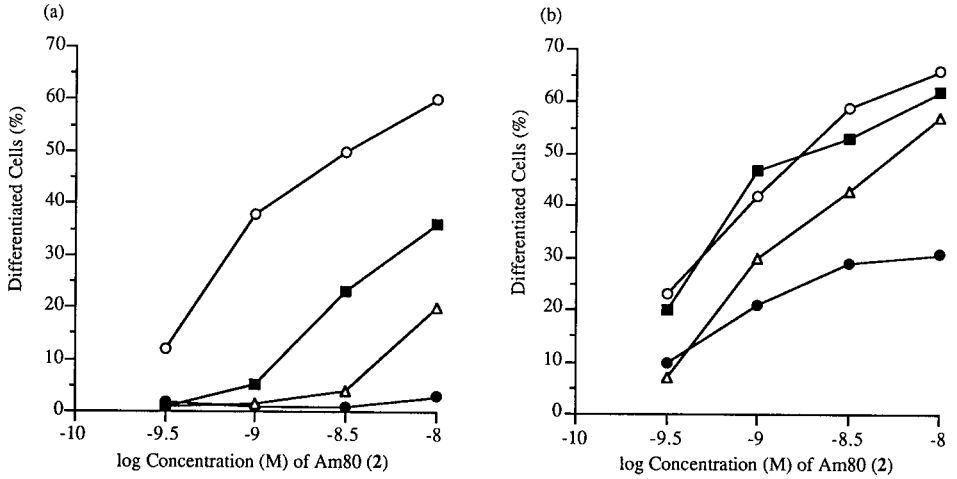


Figure 1. Anatgonistic activities of diazepine derivatives LE540 (**17**, a) and LE590 (**21**, b) on Am80 (**2**)-induced HL-60 cell differentiation. The vertical scale is the percentage of differentiated cells evaluated from NBT reduction assay, and the horizontal scale is the molar concentration of Am80 (**2**). Concentrations of test compounds are 0 (○), 1.0×10^{-7} M (■), 3.0×10^{-7} M (△), and 1.0×10^{-6} M (●).

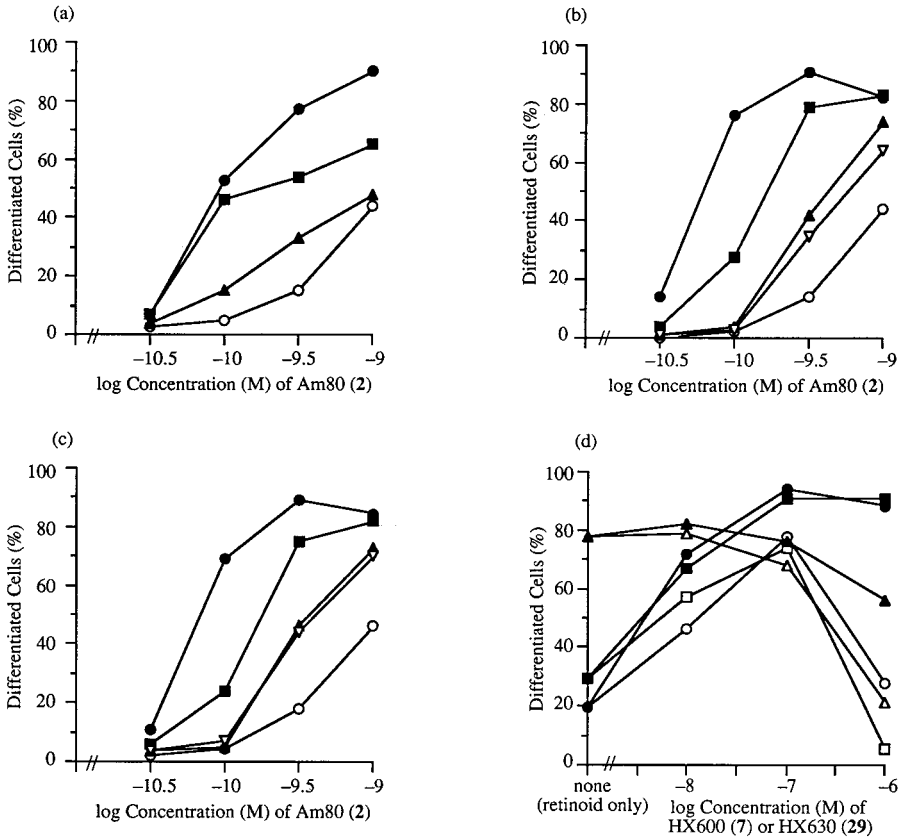


Figure 2. (a−c) Synergistic activities of HX600 (**7**), HX630 (**29**), or HX640 (**30**) with Am80 (**2**) on HL-60 cell differentiation assay. The vertical scale is the percentage of differentiated cells evaluated from NBT reduction assay, and the horizontal scale is the molar concentration of Am80 (**2**). Concentration of test compounds are 0 (○), 1.0×10^{-10} M (▽), 1.0×10^{-9} M (▲), 1.0×10^{-8} M (■), and 1.0×10^{-7} M (●). (d) Effects of HX600 (**7**, open symbol) or HX630 (**29**, closed symbol) on HL-60 cell differentiation induced by various retinoids. Concentrations of added retinoids were 1.0×10^{-9} M Am555S (**3**, circular), 1.0×10^{-10} M Ch55 (**4**, square), and 1.0×10^{-6} M LGD1069 (**5**, triangle).

were dose-dependent (Figure 2). The extent of differentiation (less than 10%) induced by 1.0×10^{-10} M Am80 (**2**) was dramatically increased to 70−80% by the addition of 1.0×10^{-7} M HX630 (**29**) or HX640 (**30**). Even when 1.0×10^{-8} M HX630 (**29**) or HX640 (**30**) was added, the EC_{50} value of Am80 (**2**) increased to 1.5×10^{-10} or 1.6×10^{-10} M, respectively. The SEC_{50} values of the azepines are 6.2×10^{-10} M for HX630 (**29**) and 5.5×10^{-10} M for HX640 (**30**), which are smaller than that for HX600 (**7**, 3.2×10^{-9} M) by 1 order of magnitude.

HX641 (**31**), a methylated analogue of HX640 (**30**), exhibited synergistic activity with Am80 (**2**) below 1.0×10^{-7} M, but it is particularly noteworthy that the differentiation induced by 3.0×10^{-8} M Am80 (**2**) was suppressed to the basal level by addition of 1.0×10^{-6} M HX641 (**31**). A similar but slight decrease of retinoid synergism was also seen reproducibly in the case of

Table 2. Synergistic Activities of Azepine Derivatives with Am80 (**2**) on HL-60 Cell Differentiation

compound	SEC_{50}, M[a]	compound	SEC_{50}, M[a]
HX600 (**7**)	3.2×10^{-9}	HX620 (**28**)	5.8×10^{-9}
HX500 (**22a**)	inactive	HX630 (**29**)	6.2×10^{-10}
HX511 (**22b**)	inactive	HX640 (**30**)	5.5×10^{-10}
HX513 (**22c**)	inactive	HX641 (**31**)	2.8×10^{-9}
HX514 (**22d**)	inactive	HX800 (**40**)	inactive
HX515 (**22e**)	1.0×10^{-7}	HX801 (**41**)	4.6×10^{-7}
HX610 (**22f**)	6.3×10^{-9}	LGD1069 (**5**)	3.1×10^{-10}

[a] SEC_{50} was determined as the concentration of a test compound which induce HL-60 cell differentiation to the extent of 50% in the presence of 3.0×10^{-10} M Am80 (**2**). This concentration of Am80 (**2**) induced the differentiation of HL-60 cells by ca. 20%. [b] "Inactive" means there was no activity below 1.0×10^{-6} M test compound.

HX600 (**7**), as reported previously,[19] as well as HX515 (**22e**) or HX610 (**22f**) at high dose (> 1.0×10^{-6} M, data not shown). At high concentrations, these compounds seem to be antagonists to RAR agonists.

Some RXR-selective retinoids have been reported to synergize with RAR ligands.[22] Thus, we compared the azepine derivatives with LGD1069 (**5**, Chart 1), a typical RXR-selective retinoid.[12] Though, in our experiments, LGD1069 (**5**) alone exhibited differentiation-inducing activity toward HL-60 cells with an EC_{50} of 2.1×10^{-7} M, LGD1069 (**5**) at lower concentration (below 1.0×10^{-7} M) enhanced the activity of Am80 (**2**), and the SEC_{50} value was 3.1×10^{-10} M. Thus, HX630 (**29**) and HX640 (**30**) are retinoid synergists as potent as RXR-selective retinoid LGD1069 (**5**).

To clarify the role of the unsubstituted benzo group of HX600 (**7**), we designed 2-oxobenzodiazepine derivatives HX800 (**40**) and HX801 (**41**, Scheme 3) in which the anilino group of HX600 (**7**) was replaced with an acetamido moiety. Only HX801 (**41**) exhibited significant synergism with Am80 (**2**), although its potency (SEC_{50}: 4.6×10^{-7} M) is weaker than that of HX600 (**7**). This result indicated that the hydrophobic properties of the unsubstituted benzo group of HX600 (**7**) were not necessary, but were important for the synergistic activity.

Effects of HX600 and HX630 on Various Retinoids. Various azepine derivatives (HX series) enhance the activity of Am80 (**2**). Previously, we reported that HX600 (**7**) also synergizes with retinoic acid (**1a**).[19] Therefore, the effects of two typical synergistic azepines, HX600 (**7**) and HX630 (**29**), on various retinoids were examined using the HL-60 assay (Figure 2d). Am555S (**3**) is a more RARα-selective retinoid than Am80 (**2**), and Ch55 (**4**) binds strongly to all three subtypes of RARs. HX600 (**7**) and HX630 (**29**) increased the potency of both retinoids in a dose-dependent manner below 1.0×10^{-7} M. HX630 (**29**) is more potent than HX600 (**7**), regardless of the retinoids.

The synergistic efficiency of HX600 (**7**) decreased when HX600 (**7**) was used at higher concentration (1.0×10^{-6} M) or when a low concentration of retinoic acid (**1a**) or Am80 (**2**) was used, as previously reported.[19] The suppressive effect at high concentration was also observed for the differentiation-inducing activity of Am555S (**3**) or Ch55 (**4**). Interestingly, the weak differentiation-inducing activity of LGD1069 (**5**) was dose-dependently inhibited by HX600 (**7**) and also slightly by HX630 (**29**). In contrast to its synergistic activity, the suppressive effect of HX600 (**7**) is more potent than that of HX630 (**29**), and the IC_{50} value of HX600 (**7**) is 4.0×10^{-7} M compared with a value of 1.0×10^{-6} M for LGD1069 (**5**).

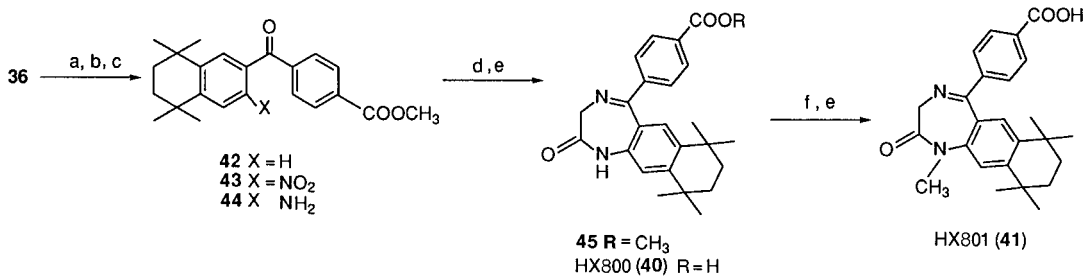
Receptor-Binding Affinities of Azepine Derivatives. The antagonistic or synergistic activities of the azepine derivatives are specific, and their structure−activity relationships indicated the possible participation of nuclear retinoid receptors. The relative binding affinities to RARs and RXRs for selected compounds were therefore examined by competition assays using recombinant receptors. The corresponding K_i values[27] are shown in Table 3 (some of these data was reported previously[15c,20,28]). The retinoid antagonist LE135 (**6**) binds selectively to RARα and RARβ; the binding affinities correlate well with its antagonistic potential in HL-60 assays. Interestingly, the binding affinities of the more potent antagonist LE540 (**17**) to RARα and RARβ are similar to those of LE135 (**6**), but LE540 (**17**) binds to all RAR and RXR subtypes with K_i values of the order of 1 μM.

Retinoid synergists (HX600 series) bind to the three RXR subtypes, but their receptor binding affinities are remarkably weaker than those of LGD1069 (**5**), and HX801 (**41**) did not significantly bind to any receptor. Furthermore, the K_i values do not correlate with their synergistic activities in HL-60 assay. LGD1069 (**5**) was reported to be an RXR-selective retinoid, but also binds to RARs with K_i values of 50−180 nM in our competitive binding experiments using recombinant receptors. HX compounds bind to RARα and RARβ, besides RXRs, but not to RARγ. Previously, we reported the dual functionality of HX600 (**6**), RAR-antagonistic and RXR-agonistic activities, based on the transactivation properties of HX600 (**6**).[20] These data indicated that the synergistic activities of HX compounds result from binding to the RXR site of RXR−RAR heterodimers, and the antagonistic activity at high doses of HX600 (**6**) results from competition at the RAR site of the heterodimers.

Discussion

Various azepine derivatives were synthesized, and their ability to regulate retinoidal activities was examined. LE135 (**6**) is a retinoid antagonist which was designed on the basis of the ligand superfamily concept.[15c] LE135 (**6**) is classified structurally as a type II antagonist (Chart 2) and is regarded as an analogue of Am80 (**2**), conformationally restricted by the bulky methylanilino group. This central aromatic moiety is apparently important for the antagonistic activity of this retinoid, since the benzodiazepine derivative with an acetamido group instead of the methylanilino group of LE135 (**6**) had no effect on retinoidal activities.[15c] The structure−activity relationships of LE135 (**6**) described above confirm the importance of this region for antagonist function, in addition to the necessary 2,5-dimethyl-2,5-hexano group. Elongation of the *N*-alkyl group did not change the antagonistic activity. The naphtho group is most effective in this series, and LE540 (**17**) is the most active retinoid antagonist among the diaryldiazepine derivatives examined. The antagonistic activity of LE540 (**17**) may be elicited through binding to RARs, as is apparently the case for LE135 (**6**). The RAR pan-antagonistic property of LE540 (**17**) is supported by the results of transactivation assay using reporter cells

Scheme 3[a]



[a] (a) $AlCl_3$/*p*-$CH_3OOCPhCOCl$; (b) KNO_3/H_2SO_4; (c) Fe/HCl; (d) $Cl^-H_3N^+CH_2COOCH_3$/pyridine; (e) NaOH/EtOH; (f) NaH; CH_3I.

Table 3. Receptor Binding Affinities of Retinoid Antagonists and Retinoid Synergists

compound	K_i values, $\times 10^{-9}$ M					
	RARα	RARβ	RARγ[a]	RXRα	RXRβ	RXRγ
			Agonist			
retinoic acid (**1a**)	3.3	4.1	0.04	330	54	120
9-*cis*-retinoic acid (**1b**)	no data	no data	1.0	12	3.8	11
Am80 (**2**)	6.5	30	nb[b]	nb	nb	nb
			RXR−Selective			
LGD1069 (**5**)	180	50	130	16	5.9	8.3
			Retinoid Antagonist			
LE135 (**6**)	1400	220	nb	nb	nb	nb
LE540 (**17**)	1300	500	490	3800	1700	1500
			Retinoid Synergist			
HX600 (**7**)	nb	680	nb	1900	640	1000
HX620 (**28**)	980	320	nb	3800	1200	2100
HX630 (**29**)	900	320	nb	900	400	620
HX640 (**30**)	1600	810	nb	1800	790	1000
HX641 (**31**)	2000	no data	nb	900	150	170
HX801 (**41**)	nb	nb	nb	nb	nb	nb

[a] See ref 27. [b] nb (not bound) means that 1000-fold excess of a test compound did not affect the binding of the labeled compound to the receptors.

stably transfected with GAL−RAR chimeras (data not shown). The subtype selectivity (RARβ > RARα ≫ RARγ) of LE135 (**6**) was less pronounced for LE540 (**17**), and the in vitro binding affinities to RARs could not explain the difference in potency between LE135 (**6**) and LE540 (**17**). The ability of LE540 (**17**) to bind to RXRs might significantly contribute to its activity.

Several azepines enhanced the activity of retinoids in HL-60 assay. These compounds are regarded as pure synergists, since they are completely inactive alone, which contrasts with the agonistic/synergistic activity of LGD1069 (**5**). In HX600 (**7**) the loss of the 2,5-dimethyl-2,5-hexano group [giving HX500 (**22a**)] or the carboxyl group (not shown) diminishes the synergistic activity. The bulky hydrophobic group can be replaced with acyclic alkyl group(s), such as diisopropyl groups, as seen in HX610 (**22f**). Replacement of one of the two nitrogen atoms of HX600 (**7**) with a nonpolar thio or methylene group increased the synergistic activity.

The synergistic activities of HX600 (**7**) could be attributed to its RXR-agonistic properties, as deduced from the receptor-binding affinities and the results of transactivation experiments.[20] However, the in vitro binding affinities of HX compounds to RXRs are very weak, with K_i values of micromolar order, as shown in Table 3. The order of the retinoid-synergistic potency of HX compounds did not reflect their K_i values to recombinant RXRs. HX630 (**29**) and HX640 (**30**) were almost as active as LGD1069 (**5**) in HL-60 differentiation assay, but their affinities to RXRs are lower than those of LGD1069 (**5**) by 2 orders of magnitude. Some ligands are reported to have different in vitro and in vivo receptor-binding affinities, as was recently suggested for a mutant glucocorticoid receptor.[29] Along the same lines, it is possible that HX compounds interact efficiently with RXRs of RXR−RAR heterodimers within the transcriptionally active complex formed with coactivators, while they may not, or very weakly, interact with RXR monomers or the homodimers in the in vitro binding assays.

HX600 (**7**) has a dual function, exerting both synergistic and antagonistic activities. Some compounds, especially those harboring an *N*-methyl group on the diazepine ring, such as HX515 (**22e**) and HX610 (**22f**), exhibited similar dual activities. This property is more marked in HX641 (**31**), having a methyl group at the corresponding benzylic position, than in HX640 (**30**) without the methyl group. The antagonistic activity at high concentrations may be mediated by binding to and inactivating RARs. HX600 (**7**), and even HX630 (**29**), suppressed the differentiation-inducing activity of the RXR-selective retinoid LGD1069 (**5**). Notably, in our experiments LGD1069 (**5**) did bind weakly to RARs. Thus LGD1069 (**5**) acts as a weak RAR agonist whose activity in the RXR−RAR heterodimer is further enhanced by its potent RXR agonistic activity. HX600 (**7**) may antagonize LGD1069 (**5**) via the RAR subunit of RXR−RAR heterodimers. The K_i values of HX compounds to RARs seem not to be simply correlated with their antagonistic activity (or reduced synergistic activity). HX630 (**29**) and HX640 (**30**) seem to be more efficient synergists than HX600 (**7**, synergist/antagonist) or even LGD1069 (**5**, synergist/agonist), considering both the potency and the selectivity of the dual func-

tions. Further structure−activity investigation is needed in order to separate RAR-antagonistic and RXR-agonistic activities.

RXRs act as heterodimer partners not only with RARs, but also with other nuclear receptors such as vitamin D_3 receptors, thyroid hormone receptors, and peroxisome proliferative activated receptors (PPARs).[7] The allosteric effects of RXR ligands in RXR−RAR heterodimers cause unique modulation of retinoid actions depending on the combinations of RAR or RXR ligands,[8c,22,30] and some of the above hormonal activities can be positively or negatively regulated by RXR ligands.[31] The biological effects of RXR-agonistic diazepines are being further evaluated in our laboratory.

In conclusion, we have developed new RAR antagonists and RXR agonists (retinoid synergists). Since they have weak in vitro binding affinities to recombinant monomeric or homodimeric retinoid nuclear receptors, but exhibit potent regulation of retinoids in HL-60 assay, HX600 (**7**) and the related synergists are RXR agonists selective for RXR−RAR heterodimers. Among retinoid synergists, HX630 (**29**) and HX640 (**30**) showed weaker RAR-antagonistic and greater RXR-agonistic activities than HX600 (**7**). These synergistic azepines should be useful tools for the elucidation of RXR functions in retinoidal and other hormonal actions. Furthermore, they can potentiate retinoid action at low concentration, and thus may find application in the field of retinoid therapy.

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 a JEOL JNM-GX400 (400 MHz) spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer.

***N*-Methyl-*N*-(1-naphthyl)-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene-2,3-diamine (26).** A mixture of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-3-nitro-2-naphthylamine (**23**, 16.4 g, 66.1 mmol), 1-bromonaphthalene (23.2 mL), potassium carbonate (9.13 g, 66.1 mmol), and copper iodide (575 mg) in *o*-xylene (180 mL) was heated at 150 °C for 19 h. After removal of the solvent, the residue was purified by silica gel column chromatography (*n*-hexane, then AcOEt:*n*-hexane, 1:40) to give **24** (66%, orange crystals, mp 127 °C, Anal. $C_{24}H_{26}N_2O_2$). A solution of **24** (24.14 g, 64.5 mmol) in DMF (400 mL) was added to a suspension of NaH (60%, 4.9 g, 129 mmol) in DMF (15 mL). After 1 h, methyl iodide (16.3 mL) was added, and the mixture was stirred for 1 h. Removal of the solvent afforded a residue, which was taken up in water and extracted with CH_2Cl_2. The organic layer was washed with water and brine and dried over Na_2SO_4. Removal of the solvent under vacuum and recrystallization from *n*-hexane gave **25** (98%, mp 154 °C, Anal. $C_{25}H_{28}N_2O_2$). **25** (3.30 g, 8.51 mmol) was dissolved in 300 mL of ethanol and was hydrogenated over 10% Pd−C (400 mg) at 40 °C for 4 h. After filtration and removal of the solvent, the residue was chromatographed on silica gel (AcOEt:*n*-hexane, 1:7) to give **26** (86%). **26**: ^{1}H NMR ($CDCl_3$) δ 8.01 (d, 1 H, J = 8.4 Hz), 7.83 (d, 1 H, J = 7.3 Hz), 7.55 (d, 1 H, J = 8.4 Hz), 7.40 (m, 3 H), 7.08 (d, 1 H, J = 7.3 Hz), 6.85 (s, 1 H), 6.64 (s, 1 H), 3.60 (brs, 2 H), 3.25 (s, 3 H), 1.61 (m, 4 H), 1.24 (s, 6 H), 1.10 (s, 6 H).

Methyl 4-[[5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-3-[*N*-methyl-*N*-(1-naphthyl)amino]-2-naphthyl]carbamoyl]benzoate (27). Terephthalic acid monomethyl ester chloride (1.88 g, 9.51 mmol) was added to a solution of **26** (2.60 g, 7.26 mmol) in dry benzene (50 mL) and pyridine (2.8 mL), and the whole was stirred for 24 h. The mixture was poured into 2 N hydrochloric acid, and extracted with AcOEt. The organic layer was dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (flash column, AcOEt:*n*-hexane, 1:20) to give **27** (95%) as colorless crystals. **27**: ^{1}H NMR ($CDCl_3$) δ 8.36 (s, 1 H), 8.22 (d, 2 H, J = 8.4 Hz), 8.07 (s, 1 H), 7.85 (m, 1 H), 7.66 (d, 2 H, J = 8.4 Hz), 7.57 (d, 1 H, J = 8.1 Hz), 7.50 (m, 2 H), 7.33 (m, 2 H), 7.25 (s, 1 H), 7.02 (m, 3 H), 3.91 (s, 3 H), 3.33 (s, 3 H), 1.73 (s, 4 H), 1.34 (s, 6 H), 1.31 (s, 6 H).

4-(13*H*-10,11,12,13-Tetrahydro-10,10,13,13,15-pentamethyldinaphtho[2,3-*b*][1,2-*e*][1,4]diazepin-7-yl)benzoic Acid (LE540, 17). A solution of **27** (4.00 g, 7.69 mmol) in a small amount of CH_2Cl_2 was added to polyphosphoric acid (40 g), and the mixture was heated at 110 °C for 2 h and then allowed to cool. Water was added, and the whole was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The crude product was purified by silica gel column chromatography (AcOEt:*n*-hexane, 1:8) to give methyl 4-(13*H*-10,11,12,13-tetrahydro-10,10,13,13,15-pentamethyldinaphtho[2,3-*b*][1,2-*e*][1,4]diazepin-7-yl)benzoate (83%), which was hydrolyzed in 2 N NaOH/ethanol at reflux for 30 min to afford LE540 (**17**, quant.). LE540 (**17**): yellow needles (aqueous ethanol); mp >300 °C; ^{1}H NMR ($CDCl_3$) δ 8.87 (d, 1 H, J = 8.4 Hz), 8.20 (d, 2 H, J = 8.4 Hz), 7.95 (d, 2 H, J = 8.8 Hz), 7.85 (d, 1 H, J = 7.7 Hz), 7.65 (m, 3 H), 7.42 (s, 1 H), 7.30 (s, 1 H), 7.17 (d, 1 H, J = 8.4 Hz), 3.08 (s, 3 H), 1.68 (s, 4 H), 1.38 (s, 3 H), 1.36 (s, 3 H), 1.22 (s, 3 H), 1.21 (s, 3 H). Anal. ($C_{33}H_{32}N_2O_2$) C, H, N.

Other diazepine derivatives (**14**, **15**, **18**−**22**) were synthesized from the corresponding nitroanilines and aryl halides according to the above method, and their chemical and physical properties are listed in Table 4.

4-(5*H*-7,8,9,10-Tetrahydro-5,7,7,10,10-pentamethyl-2-nitrobenzo[*e*]naphtho[2,3-*b*][1,4]diazepin-13-yl)benzoic Acid (LE531, 16). KNO_3 (18.4 mg, 0.18 mmol) was added to a solution of methyl 4-(5*H*-7,8,9,10-tetrahydro-5,7,7,10,10-pentamethylbenzo[*e*]naphtho[2,3-*b*][1,4]diazepin-13-yl)benzoate (methyl ester of LE135, 60 mg, 0.132 mmol) in sulfuric acid (5 mL) at 0 °C, and the mixture was stirred at 0 °C for 1 h, then poured into ice water, and extracted with CH_2Cl_2. The organic layer was washed successively with 1 N $NaHCO_3$, water, and brine and dried over Na_2SO_4. After evaporation, the residue was chromatographed on silica gel (flash column, AcOEt:*n*-hexane, 1:8) to give methyl 4-(5*H*-7,8,9,10-tetrahydro-5,7,7,10,10-pentamethyl-2-nitrobenzo[*e*]naphtho[2,3-*b*][1,4]-diazepin-13-yl)benzoate (56%), which was hydrolyzed in 2 N NaOH/ethanol at reflux for 1 h to afford LE531 (**16**, 71%). LE531 (**16**): orange needles (aqueous ethanol); mp >300 °C; ^{1}H NMR ($CDCl_3$) δ 8.27 (dd, 1 H, J = 8.4, 2.0 Hz), 8.15 (d, 2 H, J = 8.8 Hz), 7.83 (d, 1 H, J = 8.4 Hz), 7.80 (d, 2 H, J = 8.4 Hz), 7.25 (s, 1 H), 7.13 (d, 1 H, J = 2.0 Hz), 6.84 (s, 1 H), 3.34 (s, 3 H), 1.67 (s, 4 H), 1.32 (s, 3 H), 1.26 (s, 9 H). Anal. ($C_{29}H_{29}N_3O_4 \cdot {}^1/_3\ H_2O$), C, H, N.

2-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)oxy]aniline (34). A mixture of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthol (97 mg, 0.475 mmol), 1-chloro-2-nitrobenzene (77 mg, 0.48 mmol), and potassium hydroxide (27 mg, 0.48 mmol) in DMSO (5 mL) was heated at 90 °C for 17.5 h. The reaction mixture was poured into 2 N hydrochloric acid and extracted with CH_2Cl_2. The organic layer was washed with 2 N hydrochloric acid and brine and dried over Na_2SO_4. After evaporation, the crude product was chromatographed on silica gel (flash column, AcOEt:*n*-hexane, 1:30) to give 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-(2-nitrophenoxy)naphthalene (67%) as a colorless oil. Fe powder (220 mg) was added to a suspension of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-(2-nitrophenoxy)naphthalene (103 mg) in 2 N hydrochloric acid (2.5 mL) and ethanol (6 mL), and the mixture was refluxed for 30 min. The reaction mixture was filtered, and the filtrate was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated to give crude **34**. **34**: colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.21 (d, 1 H, J = 8.8 Hz), 6.97 (d, 1 H, J = 2.9 Hz), 6.95 (m, 1 H), 6.85 (dd, 1 H, J = 8.1, 1.5 Hz), 6.82 (dd, 1 H, J = 7.7, 1.5 Hz), 6.70 (m, 2 H), 3.82 (brs, 2 H), 1.68 (s, 4 H), 1.26 (s, 6 H), 1.25 (s, 6 H).

Table 4. Chemical and Physical Properties of the Azepine Derivatives

compound	mp, °C	crystal form	recrystn solvent	anal. formula
LE510 (**14a**)	172	yellow needles	EtOH−H_2O	$C_{27}H_{28}N_2O_2$
LE511 (**14b**)	266	orange needles	EtOH−H_2O	$C_{25}H_{24}N_2O_2$
LE515 (**14c**)	298	yellow needles	EtOH−H_2O	$C_{31}H_{30}N_2O_2 \cdot {}^1/_2H_2O$
LE523 (**15a**)	191	orange needles	EtOH−H_2O	$C_{31}H_{34}N_2O_2 \cdot {}^1/_2H_2O$
LE527 (**15b**)	128	orange needles	EtOH−H_2O	$C_{35}H_{42}N_2O_2$
LE531 (**16**)	>300	orange needles	EtOH−H_2O	$C_{29}H_{29}N_3O_4 \cdot {}^1/_3H_2O$
LE540 (**17**)	>300	yellow needles	EtOH−H_2O	$C_{33}H_{32}N_2O_2$
LE550 (**18**)	>300	red cubes	EtOH−H_2O	$C_{33}H_{32}N_2O_2 \cdot {}^1/_3H_2O$
LE560 (**19**)	>300	yellow needles	EtOH−H_2O	$C_{37}H_{34}N_2O_2$
LE570 (**20**)	283−285	orange needles	EtOH−H_2O	$C_{37}H_{34}N_2O_2 \cdot {}^2/_3H_2O$
LE590 (**21**)	289	yellow needles	EtOH−H_2O	$C_{37}H_{44}N_2O_2$
HX500 (**22a**)	246	orange needles	EtOH−H_2O	$C_{21}H_{16}N_2O_2$
HX511 (**22b**)	249	orange needles	EtOH−H_2O	$C_{25}H_{24}N_2O_2$
HX513 (**22c**)	294−295	orange prisms	MeOH	$C_{21}H_{15}N_2O_2Br$
HX514 (**22d**)	247	orange needles	EtOH−H_2O	$C_{27}H_{20}N_2O_2$
HX515 (**22e**)	208	orange needles	EtOH−H_2O	$C_{31}H_{30}N_2O_2 \cdot {}^1/_3H_2O$
HX610 (**22f**)	263	orange needles	EtOH−H_2O	$C_{27}H_{28}N_2O_2$
HX620 (**28**)	289	yellow needles	EtOH−H_2O	$C_{28}H_{27}NO_3$
HX630 (**29**)	299	yellow needles	EtOH−H_2O	$C_{28}H_{27}NO_2S$
HX640 (**30**)	>300	yellow needles	EtOH−H_2O	$C_{29}H_{29}NO_2$
HX641 (**31**)	224−226	yellow prisms	EtOH−H_2O	$C_{30}H_{31}NO_2 \cdot {}^3/_4H_2O$
HX800 (**40**)	>300	colorless needles	MeOH−*n*-hexane	$C_{24}H_{26}N_2O_3 \cdot {}^3/_4H_2O$
HX801 (**41**)	>300	colorless needles	AcOEt−*n*-hexane	$C_{25}H_{28}N_2O_3$

4-[2,3-(2,5-Dimethyl-2,5-hexano)dibenz[*b,f*][1,4]-oxazepin-11-yl]benzoic Acid (HX620, 28). Terephthalic acid monomethyl ester chloride (63 mg, 0.32 mmol) was added to a solution of **34** (80.5 mg, 0.264 mmol) in dry benzene (5 mL) and pyridine (0.1 mL), and the whole was stirred for 16.5 h. The mixture was poured into 2 N hydrochloric acid and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (flash column, AcOEt:*n*-hexane 1:20) to give methyl 4-[[2-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)oxy]phenyl]carbamoyl]benzoate (94%). A mixture of methyl 4-[[2-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-oxy]phenyl]carbamoyl]benzoate (111 mg, 0.238 mmol) and polyphosphoric acid (2.2 g) was heated at 100 °C for 1.5 h. After cooling, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (flash column, AcOEt:*n*-hexane, 1:40) to give methyl 4-[2,3-(2,5-dimethyl-2,5-hexano)dibenz[*b,f*][1,4]oxazepin-11-yl]benzoate (31%), which was hydrolyzed in 2 N NaOH/ethanol for 40 min to afford HX620 (**28**, quant.). HX620 (**28**): yellow needles (aqueous ethanol); mp 289 °C; ^{1}H NMR ($CDCl_3$) δ 8.19 (d, 2 H, J = 8.8 Hz), 7.97 (d, 2 H, J = 8.8 Hz), 7.46 (m, 1 H), 7.22 (m, 3 H), 7.18 (s, 1 H), 7.02 (s, 1 H), 1.66 (s, 4 H), 1.31 (s, 6 H), 1.12 (s, 6 H). Anal. ($C_{28}H_{27}NO_3$) C, H, N.

2-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-thio]aniline (35). A mixture of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenethiol (4.0 g, 18.2 mmol), 1-chloro-2-nitrobenzene (4.30 g, 27.3 mmol), and potassium hydroxide (1.0 g, 18.2 mmol) in DMSO (60 mL) was heated at 100 °C for 21 h. The reaction mixture was concentrated under vacuum and then poured into 2 N hydrochloric acid and extracted with CH_2Cl_2. The organic layer was washed with 2 N hydrochloric acid and brine and dried over Na_2SO_4. After evaporation, the crude product was chromatographed on silica gel (AcOEt:*n*-hexane, 1:25) to give 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-[(2-nitrophenyl)thio]naphthalene (66%, orange crystals from *n*-hexane, mp 107 °C, Anal. $C_{20}H_{23}NO_2S$). Fe powder (2.9 g) was added to a suspension of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-[(2-nitrophenyl)thio]naphthalene (3.85 g, 11.3 mmol) in 2 N hydrochloric acid (18 mL) and ethanol (30 mL), and the mixture was refluxed for 15 min. The reaction mixture was filtered, and the filtrate was extracted with AcOEt. The organic layer was washed with water and brine and then dried over Na_2SO_4. After evaporation, the residue was chromatographed on silica gel (AcOEt:*n*-hexane, 1:20) to give **35** (38%). **35**: colorless prisms (EtOH−H_2O); mp 114 °C; ^{1}H NMR ($CDCl_3$) δ 7.43 (dd, 1 H, J = 7.5, 1.8 Hz), 7.21 (dt, 1 H, J = 7.3, 1.4 Hz), 7.14 (d, 1 H, J = 8.1 Hz), 7.10 (d, 1 H, J = 2.2 Hz), 6.77 (m, 3 H), 4.30 (brs, 2 H), 1.64 (s, 4 H), 1.22 (s, 6 H), 1.20 (s, 6 H). Anal. ($C_{20}H_{25}NS$) C, H, N.

4-[2,3-(2,5-Dimethyl-2,5-hexano)dibenzo[*b,f*][1,4]-thiazepin-11-yl]benzoic Acid (HX630, 29). Terephthalic acid monomethyl ester chloride (1.10 g, 5.52 mmol) was added to a solution of **35** (1.32 g, 4.24 mmol) in dry benzene (265 mL) and pyridine (4.2 mL), and the mixture was stirred for 6 h. The reaction mixture was poured into 2 N hydrochloric acid and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (AcOEt:*n*-hexane, 1:8) to give methyl 4-[[2-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)thio]-phenyl]carbamoyl]benzoate (91%, colorless prisms from *n*-hexane, mp 137 °C, Anal. $C_{29}H_{31}NO_3S$). A mixture of methyl 4-[[2-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)thio]-phenyl]carbamoyl]benzoate (1.83 g, 3.87 mmol, dissolved in a small amount of CH_2Cl_2) and polyphosphoric acid (12 g) was heated at 110 °C for 50 min. After cooling, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (AcOEt:*n*-hexane, 1:8) to give methyl 4-[2,3-(2,5-dimethyl-2,5-hexano)dibenzo-[*b,f*][1,4]thiazepin-11-yl]benzoate (89%, yellow crystal from EtOH−H_2O, mp 221 °C, Anal. $C_{29}H_{29}NO_2S$), which was hydrolyzed in 2 N NaOH/ethanol for 40 min to afford HX630 (**29**, 97%). HX630 (**29**): yellow needles (EtOH−H_2O); mp 299 °C; ^{1}H NMR ($CDCl_3$) δ 8.17 (d, 2 H, J = 8.4 Hz), 7.94 (d, 2 H, J = 8.4 Hz), 7.48 (dd, 1 H, J = 7.7, 1.1 Hz), 7.45 (s, 1 H), 7.37 (m, 2 H), 7.13 (m, 1 H), 7.04 (s, 1 H), 1.65 (m, 4 H), 1.31 (s, 3 H), 1.28 (s, 3 H), 1.15 (s, 3 H), 1.07 (s, 3 H). Anal. ($C_{28}H_{27}NO_2S$) C, H, N.

5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-(2-nitrobenzoyl)naphthalene (37). $AlCl_3$ (14.3 g) was added portionwise to a mixture of 1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (**36**, 10.0 g, 53.2 mmol) and 2-nitrobenzoyl chloride (9.4 g, 50.5 mmol) in dry CH_2Cl_2 (50 mL), and the mixture was refluxed for 1.5 h. The reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated. The crude product was purified by silica gel column chromatography (flash column, AcOEt:*n*-hexane, 1:10) to give **37** (42%). **37**: colorless needles (*n*-hexane); mp 122 °C; ^{1}H NMR ($CDCl_3$) δ 8.23 (d, 1 H, J = 8.1 Hz), 7.84 (s, 1 H), 7.75 (t, 1 H, J = 6.2 Hz), 7.69 (t, 1 H, J = 7.0 Hz), 7.48 (dd, 1 H, J = 7.7, 1.5 Hz), 7.34 (m, 2 H), 1.69 (s, 4 H), 1.28 (s, 6 H), 1.26 (s, 6 H). Anal. ($C_{21}H_{23}NO_3$) C, H, N.

4-[2,3-(2,5-Dimethyl-2,5-hexano)dibenz[*b,e*]azepin-11-yl]benzoic Acid (HX640, 30). Fe powder (313 mg) was added to a suspension of **37** (262 mg, 0.78 mmol) in hydrochloric acid (2 mL) and ethanol (10 mL), and the mixture was refluxed for

15 min. The reaction mixture was filtered, and the filtrate was extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated to give crude 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthoyl)aniline (quant.). A solution of 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthoyl)aniline (67 mg, 0.22 mmol) in ether (2 mL) was added to a suspension of $LiAlH_4$ (41 mg, 1.09 mmol) in ether (8 mL), and the mixture was refluxed for 19 h, then poured into water, and extracted with ether. The organic layer was dried over Na_2SO_4 and evaporated. The crude product was chromatographed on silica gel (flash column, AcOEt:*n*-hexane, 1:40 then 1:20) to give **38** (54%). Terephthalic acid monomethyl ester chloride (74 mg, 0.37 mmol) was added to a solution of **38** (88.5 mg, 0.30 mmol) in dry benzene (4 mL) and pyridine (0.2 mL), and the mixture was stirred for 1.5 h, poured into 2 N hydrochloric acid, and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (flash column, AcOEt:*n*-hexane, 1:20) to give methyl 4-[[2-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)methyl]phenyl]carbamoyl]benzoate (84%). A mixture of methyl 4-[[2-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)methyl]phenyl]carbamoyl]benzoate (103 mg, 0.23 mmol) and polyphosphoric acid (1.56 g) was heated at 110 °C for 45 min. After cooling, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (flash column, AcOEt:*n*-hexane, 1:20) to give methyl 4-[2,3-(2,5-dimethyl-2,5-hexano)dibenz[*b*,*e*]azepin-11-yl]benzoate (79%), which was hydrolyzed in 2 N NaOH/ethanol for 1 h to afford HX640 (**30**, 97%). HX640 (**30**): yellow needles (EtOH−H_2O); mp >300 °C; ^{1}H NMR (DMSO-d_6, 120 °C) δ 8.05 (d, 2 H, J = 8.4 Hz), 7.89 (d, 2 H, J = 8.4 Hz), 7.39 (s, 1 H), 7.33 (m, 2 H), 7.26 (td, 1 H, J = 7.3, 1.5 Hz), 7.16 (td, 1 H, J = 7.3, 1.5 Hz), 7.09 (s, 1 H), 3.69 (s, 2 H), 1.66 (m, 4 H), 1.32 (s, 6 H), 1.11 (s, 6 H). Anal. ($C_{29}H_{29}NO_2$) C, H, N.

2-[1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)ethyl]aniline (39). *n*-BuLi (1.6 M in hexane, 1.25 mL) was added to a suspension of methyltriphenylphosphonium bromide (542 mg, 1.50 mmol) in THF (5 mL) at −50 °C. After 15 min, a solution of **37** (337 mg, 1.00 mmol) in THF (5 mL) was added to the solution of the ylide, and the whole was held at room temperature overnight, then poured into water, and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (flash column, AcOEt:*n*-hexane, 1:20) to give 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-[1-(2-nitrophenyl)-ethenyl]naphthalene (79%), which was hydrogenated over 10% Pd−C in methanol for 2 h to afford **39** (78%). **39**: ^{1}H NMR ($CDCl_3$) δ 7.27 (d, 1 H, J = 7.7 Hz), 7.17 (m, 2 H), 7.07 (td, 1 H, J = 7.7, 1.5 Hz), 6.86 (m, 2 H), 6.65 (d, 1 H, J = 7.7 Hz), 4.03 (q, 1 H, J = 7.3 Hz), 3.60 (brs, 2 H), 1.65 (s, 4 H), 1.61 (d, 3 H, J = 7.0 Hz), 1.25 (s, 3 H), 1.24 (s, 3 H), 1.23 (s, 3 H), 1.22 (s, 3 H).

4-[2,3-(2,5-Dimethyl-2,5-hexano)-5-methyldibenz[*b*,*e*]-azepin-11-yl]benzoic Acid (HX641, 31). Terephthalic acid monomethyl ester chloride (97 mg, 0.49 mmol) was added to a solution of **39** (121 mg, 0.40 mmol) in dry benzene (8 mL) and pyridine (0.2 mL), and the mixture was stirred for 15 h. The reaction mixture was poured into 2 N hydrochloric acid and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (flash column, AcOEt:*n*-hexane, 1:20) to give methyl 4-[[2-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)ethyl]phenyl]carbamoyl]benzoate (78%). A mixture of methyl 4-[[2-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)ethyl]phenyl]carbamoyl]benzoate (140 mg, 0.30 mmol) and polyphosphoric acid (1.20 g) was heated at 150 °C for 40 min. After cooling, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (flash column, AcOEt:*n*-hexane, 1:40) to give methyl 4-[2,3-(2,5-dimethyl-2,5-hexano)-5-methyldibenz[*b*,*e*]-azepin-11-yl]benzoate (49%), which was hydrolyzed in 2 N NaOH/ethanol for 3 h to afford HX641 (**31**, 91%). HX641 (**31**): yellow needles (aqueous ethanol); mp >300 °C; ^{1}H NMR ($CDCl_3$, two conformers) δ 8.19 (d, 1 H, J = 8.4 Hz), 8.18 (d, 1 H, J = 8.4 Hz), 7.99 (d, 1 H, J = 8.4 Hz), 7.97 (d, 1 H, J = 8.4 Hz), 7.46 (br, 1 H), 7.25 (m, 4 H), 7.10 (s, 0.5 H), 7.09 (s, 0.5 H), 4.03 (q, 0.5 H, J = 7.3 Hz), 3.62 (q, 0.5 H, J = 7.3 Hz), 1.85 (d, 1.5 H, J = 7.3 Hz), 1.65 (m, 4 H), 1.37 (s, 1.5 H), 1.37 (s, 1.5 H), 1.34 (d, 1.5 H, J = 7.3 Hz), 1.27 (s, 1.5 H), 1.26 (s, 1.5 H), 1.17 (s, 1.5 H), 1.16 (s, 1.5 H), 1.04 (s, 1.5 H), 1.03 (s, 1.5 H); HRMS calcd for $C_{30}H_{31}NO_2$ 437.2356, Found: 437.2385. Anal. ($C_{30}H_{31}NO_2 \cdot {}^3/_4H_2O$) C, H, N.

Methyl 4-(5,5,8,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthoyl)benzoate (42). $AlCl_3$ (14.3 g, 107.5 mmol) was added portionwise to a mixture of 1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (**36**, 10.0 g, 53.2 mmol) and terephthalic acid monomethyl ester chloride (10.0 g, 50.5 mmol) in CH_2Cl_2 (50 mL) at 0 °C, and the whole was refluxed for 1 h. The reaction mixture was poured into ice water and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. After evaporation, the crude product was recrystallized from AcOEt to give **42** (99%). **42**: colorless needles (AcOEt); mp 139 °C; ^{1}H NMR ($CDCl_3$) δ 8.15 (d, 2 H, J = 8.8 Hz), 7.83 (d, 2 H, J = 8.4 Hz), 7.79 (d, 1 H, J = 1.8 Hz), 7.54 (dd, 1 H, J = 8.1, 1.8 Hz), 7.41 (d, 1 H, J = 8.4 Hz), 3.97 (s, 3 H), 1.72 (s, 4 H), 1.32 (s, 6 H), 1.29 (s, 6 H). Anal. ($C_{23}H_{26}O_3$) C, H, N.

Methyl 4-(5,5,8,8-Tetrahydro-5,5,8,8-tetramethyl-3-nitro-2-naphthoyl)benzoate (43). KNO_3 (240 mg, 2.37 mmol) was added to a solution of **42** (693 mg, 1.98 mmol) in sulfuric acid (5 mL) at 0 °C, and the mixture was stirred for 1 h. The reaction mixture was poured into ice water and extracted with CH_2Cl_2. The organic layer was washed with 1 N $NaHCO_3$ and brine and dried over Na_2SO_4. After evaporation, the crude product was recrystallized from AcOEt to give **43** (53%). **43**: colorless needles (AcOEt); mp 192 °C; ^{1}H NMR ($CDCl_3$) δ 8.16 (s, 1 H), 8.11 (d, 2 H, J = 8.4 Hz), 7.81 (d, 2 H, J = 8.4 Hz), 7.38 (s, 1 H), 3.94 (s, 3 H), 1.77 (s, 4 H), 1.39 (s, 6 H), 1.31 (s, 6 H). Anal. ($C_{23}H_{25}NO_5$) C, H, N.

Methyl 4-(3-Amino-5,5,8,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthoyl)benzoate (44). Fe powder (317 mg) was added to a suspension of **43** (318.5 mg, 0.81 mmol) in 2 N hydrochloric acid (6 mL) and ethanol (10 mL), and the mixture was refluxed for 50 min. The reaction mixture was filtered, and the filtrate was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated to afford a crude product, which was recrystallized from AcOEt:*n*-hexane to give **44** (95%). **44**: yellow needles (AcOEt:*n*-hexane); mp 162 °C; ^{1}H NMR ($CDCl_3$) δ 8.14 (d, 2 H, J = 8.4 Hz), 7.69 (d, 2 H, J = 8.8 Hz), 7.31 (s, 1 H), 6.67 (s, 1 H), 5.90 (brs, 1 H), 3.97 (s, 3 H), 1.65 (m, 4 H), 1.28 (s, 6 H), 1.11 (s, 6 H). Anal. ($C_{23}H_{27}NO_3$), C, H, N.

4-[1,3-Dihydro-7,8-(2,5-dimethyl-2,5-hexano)-2-oxo-2*H*-1,4-benzodiazepin-5-yl]benzoic Acid (HX800, 40). A mixture of **44** (70 mg, 0.19 mmol) and glycine methyl ester hydrochloride (38 mg, 0.31 mmol) in pyridine (5 mL) was refluxed for 16 h. The reaction mixture was poured into 2 N hydrochloric acid and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated to afford a residue, which was chromatographed on silica gel (flash column, AcOEt:*n*-hexane, 1:4) to give methyl 4-[1,3-dihydro-7,8-(2,5-dimethyl-2,5-hexano)-2-oxo-2*H*-1,4-benzodiazepin-5-yl]benzoate (45%). This product was hydrolyzed in 2 N NaOH/ethanol for 20 min to afford HX800 (**40**, 83%). HX800 (**40**): yellow needles (MeOH−*n*-hexane); mp >300 °C; ^{1}H NMR (DMSO-d_6, 120 °C) δ 8.05 (d, 2 H, J = 8.4 Hz), 7.89 (d, 2 H, J = 8.4 Hz), 7.39 (s, 1 H), 7.33 (m, 2 H), 7.26 (td, 1 H, J = 7.3, 1.5 Hz), 7.16 (td, 1 H, J = 7.3, 1.5 Hz), 7.09 (s, 1 H), 3.69 (s, 2 H), 1.66 (m, 4 H), 1.32 (s, 6 H), 1.11 (s, 6 H). Anal. ($C_{24}H_{26}N_2O_3 \cdot {}^3/_4H_2O$) C, H, N.

4-[1,3-Dihydro-7,8-(2,5-dimethyl-2,5-hexano)-1-methyl-2-oxo-2*H*-1,4-benzodiazepin-5-yl]benzoic Acid (HX801, 41). A solution of **45** (36 mg, 0.089 mmol) in dry DMF (4 mL) was added to a suspension of NaH (60%, 7 mg, 0.18 mmol) in DMF (1 mL). After 10 min, methyl iodide (0.02 mL) was added to the mixture, and the whole was stirred for 2.5 h and then evaporated. The residue was taken up in water and extracted with CH_2Cl_2. The organic layer was washed with water and brine and dried over Na_2SO_4. After evaporation, the residue was chromatographed on silica gel (AcOEt:*n*-hexane, 1:1) to

give methyl 4-[1,3-dihydro-7,8-(2,5-dimethyl-2,5-hexano)-1-methyl-2-oxo-2*H*-1,4-benzodiazepin-5-yl]benzoate (59%), which was hydrolyzed in 2 N NaOH/ethanol for 40 min to afford HX801 (**41**, 83%). HX801 (**41**): colorless needles (AcOEt:*n*-hexane); mp >300 °C; ^{1}H NMR ($CDCl_3$) δ 8.13 (d, 2 H, J = 8.8 Hz), 7.77 (d, 2 H, J = 8.4 Hz), 7.22 (s, 1 H), 7.14 (s, 1 H), 4.84 (d, 1 H, J = 10.6 Hz), 3.88 (d, 1 H, J = 10.6 Hz), 3.41 (s, 3 H), 1.72 (m, 4 H), 1.39 (s, 3 H), 1.32 (s, 3 H), 1.21 (s, 3 H), 1.15 (s, 3 H). Anal. ($C_{25}H_{28}N_2O_3$) C, H, N.

Cells and Culture. The human promyelocytic leukemia cell line HL-60[23,24] was provided by Prof. F. Takaku (Faculty of Medicine, University of Tokyo) in 1980, and has been maintained in continuous suspension culture.[32] The cells are cultured in plastic flasks in RPMI1640 medium, supplemented with 5% fetal bovine serum (FBS, not delipidized) and antibiotics (penicillin G and streptomycin), in a humidified atmosphere of 5% CO_2 in air at 37 °C.

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. Am80 (**2**), 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 check for morphological change. The percentages of differentiated cells were determined by nitro-blue tetrazolium (NBT) reduction assay as described.[25] Cells were incubated for 20 min at 37 °C in RPMI1640 medium (5% FBS) 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 was always consistent with the morphological result.[26]

Modification of the differentiation-inducing activity of Am80 (**2**) was examined in the presence of a suitable concentration of the test compound according to the method described above. In this experiment, the independent effects of Am80 (**2**) and the test compound were always assayed, and the percentages of differentiated cells were determined by NBT reduction assay. The assays of test compounds were performed at least twice. Synergistic effective concentration (SEC_{50}) value was defined as the EC_{50} of the synergist in combination with 3.0×10^{-10} M Am80 (**2**). IC_{50} and SEC_{50} values of active compounds were calculated from the NBT reduction assay data by means of the van der Waerden method.[33]

Competitive Binding Assay. The binding activities for RARs and RXRs were measured by the nitrocellulose filter binding assay method as described previously.[15c] Recombinant receptors were prepared as fusion proteins with the maltose-binding protein (MBP) for RARα and -β (ligand-binding domain), or with glutathione-*S*-transferase (GST) for RXRs. Recombinant RARγ was prepared as described.[28] Competitive binding assays were carried out using 8 nM [^{3}H]Am80 (65 Ci/mmol, Amersham) for RARα, 20 nM [^{3}H]Am80 for RARβ, 4 nM [^{3}H]-9-*cis*-retinoic acid (48 Ci/mmol, Amersham, used at 8 Ci/mmol by dilution with cold 9-*cis*-retinoic acid) for RARγ, or 10 nM [^{3}H]-9-*cis*-retinoic acid for RXRs. The K_i values of compounds were calculated according to the following equation: $K_i = IC_{50}/(1 + [L]/K_d)$, where IC_{50} is the concentration of competing compounds required to decrease specific binding of the labeled compound by 50% and [L] and K_d are the concentration and the dissociation constant of the labeled compound, respectively.[27,34]

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JM9704309