Retinoid X Receptor-Antagonistic Diazepinylbenzoic Acids

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Received July 26, 1999; accepted August 30, 1999

Several dibenzodiazepine derivatives were identified as novel retinoid X receptor (RXR) antagonists on the basis of inhibitory activity on retinoid-induced cell differentiation of human promyelocytic leukemia cells HL-60 and transactivation assay using retinoic acid receptors (RARs) and RXRs in COS-1 cells. 4-(5*H*-2,3-(2,5-Dimethyl-2,5-hexano)-5-*n*-propyldibenzo[*b*,*e*][1,4]diazepin-11-yl)benzoic acid (HX603, 6c) is an *N*-*n*-propyl derivative of an RXR pan-agonist HX600 (6a), and exhibited RXR-selective antagonistic activity. Similar RXR-antagonistic activities were observed with 4-(5*H*-2,3-(2,5-dimethyl-2,5-hexano)-5-methyl-8-nitrodibenzo[*b*,*e*][1,4]diazepin-11-yl)benzoic acid (HX531, 7a) and 4-(5*H*-10,11-dihydro-5,10-dimethyl-2,3-(2,5-dimethyl-2,5-hexano)-dibenzo[*b*,*e*][1,4]diazepin-11-yl)benzoic acid (HX711, 8b), which also inhibited transactivation of RARs induced by an RAR agonist, Am80. These compounds inhibited HL-60 cell differentiation induced by the combination of a low concentration of the retinoid agonist Am80 with an RXR agonist (a retinoid synergist, HX600). These results indicated that HX603 (6c), and the related RXR antagonists inhibit the activation of RAR–RXR heterodimers as well as RXR homodimers, which is a distinct characteristic different from that of the known RXR antagonist, LG100754 (9).

Key words retinoid; antagonist; retinoid X receptor; diazepine

Retinoids regulate a wide variety of biological functions, including cell differentiation, proliferation and morphogenesis.[2a)] Their biological activities are mediated by two types of specific nuclear receptors, retinoic acid receptors (RARα, β and γ) and retinoid X receptors (RXRα, β and γ).[2b,c)] These receptors, belonging to the nuclear receptor superfamily, act as ligand-inducible transcriptional factors of gene networks. RARs can bind to and be activated by both retinoic acid (all-*trans*, **1a**, Fig. 1) and 9-*cis*-retinoic acid (**1b**), while RXRs can bind to the latter isomer. Various synthetic retinoids such as Am80 (**2**) bind only to RARs with an affinity that correlates well with most retinoidal activities. Retinoid antagonists[3)] so far known, such as LE135 (**3**) and LE540 (**4**),[4,5)] also bind to RARs, and exhibit no RAR transactivation activities.

RXRs are transcriptionally silent partners of RARs, and RAR–RXR heterodimers activated by RAR ligands regulate specific gene expression.[2b,c,6)] Recently, synthetic RXR-specific ligands[7)] and their retinoidal synergistic activities have been reported.[8)] For example, RXR pan-agonists, such as LGD1069 (**5**)[9)] and HX600 (**6a**, Fig. 2), themselves exhibit little or no retinoidal activity, but strongly enhance the potencies of retinoic acid (**1a**) or Am80 (**2**).[8)] Since RXRs form heterodimers with various nuclear receptors such as vitamin D_3 receptors, thyroid hormone receptors and peroxisome proliferator-activated receptors, they may modulate the action of their ligands, besides retinoidal activities.[10)]

Interestingly, HX600 (**6a**), an isomer of an RAR antagonist LE135 (**3**), acts as an RXR agonist/retinoid synergist.[5)] During further investigations on the structure–activity relationships of retinoid-regulatory diazepinylbenzoic acids, we found that several structural modifications of the retinoid synergist HX600 (**6a**) led to novel RXR antagonists, such as HX531 (**7a**) and HX711 (**8b**), which can inhibit the activation of RAR–RXR heterodimers.[11)] This is different from the known antagonist, LG100754 (**9**), the first RXR antagonist to be discovered, which exhibits antagonistic activity towards RXR homodimers, but acts as an agonist for heterodimeric RXRs.[12)] In this paper, we describe the syntheses and biological activities of the novel RXR antagonists shown in Fig. 2.

Results

Chemistry The dibenzodiazepine derivatives, **6b—f**, **7b** and **7e**, were synthesized according to the method used for

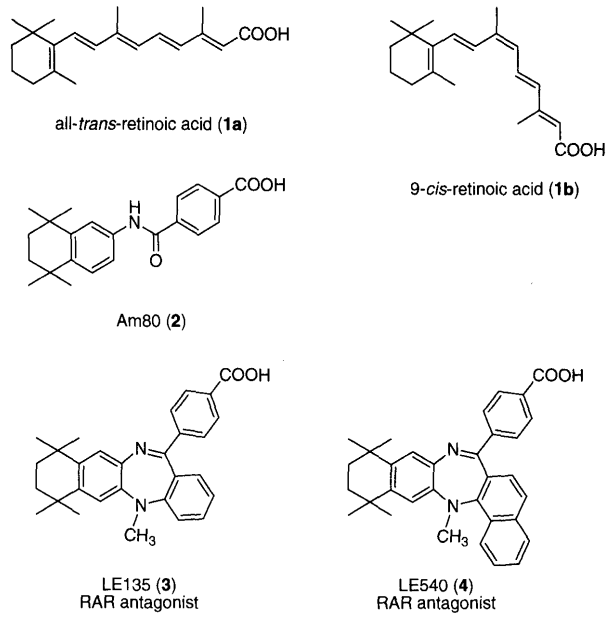

Fig. 1

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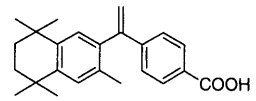
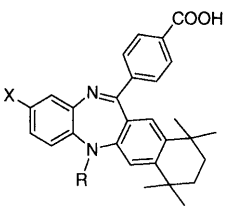
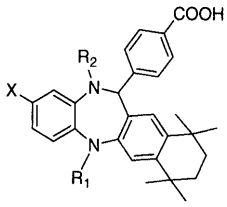
HX600 (**6a**), as shown in Chart 1.[5] *o*-Nitroaniline (**10a**) was reacted with 6-bromo-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene in the presence of copper iodide and potassium carbonate to yield a diphenylamine derivative **11a** (75%). After *N*-ethylation (quant.), **12a** was hydrogenated over Pd-C in ethanol (86%). The diamine **13a** was condensed with terephthalic acid monomethyl ester chloride (quant.), and then cyclized using polyphosphoric acid (PPA) (17%), followed by ester hydrolysis (64%), to give **6b**.



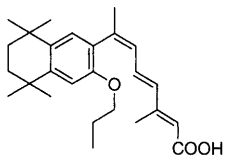
LGD1069 (**5**)
RXR agonist





	X	R_1	R_2
HX710 (**8a**)	H	CH_3	H
HX711 (**8b**)	H	CH_3	CH_3
HX741 (**8c**)	OCH_3	CH_3	CH_3
HX743 (**8d**)	OCH_3	*n*-C_3H_7	CH_3
HX745 (**8e**)	OCH_3	CH_3	*n*-C_3H_7

	X	R
HX600 (**6a**)	H	CH_3
HX602 (**6b**)	H	C_2H_5
HX603 (**6c**)	H	*n*-C_3H_7
HX604 (**6d**)	H	*n*-C_4H_9
HX605 (**6e**)	H	*n*-C_5H_{11}
HX607 (**6f**)	H	*n*-C_7H_{13}
HX531 (**7a**)	NO_2	CH_3
HX533 (**7b**)	COOH	CH_3
HX535 (**7c**)	NHAc	CH_3
HX539 (**7d**)	Br	CH_3
HX541 (**7e**)	OCH_3	CH_3
HX543 (**7f**)	OCH_3	*n*-C_3H_7
HX560 (**7g**)	Ph	CH_3



LG100754 (**9**)
RXR antagonist

Fig. 2

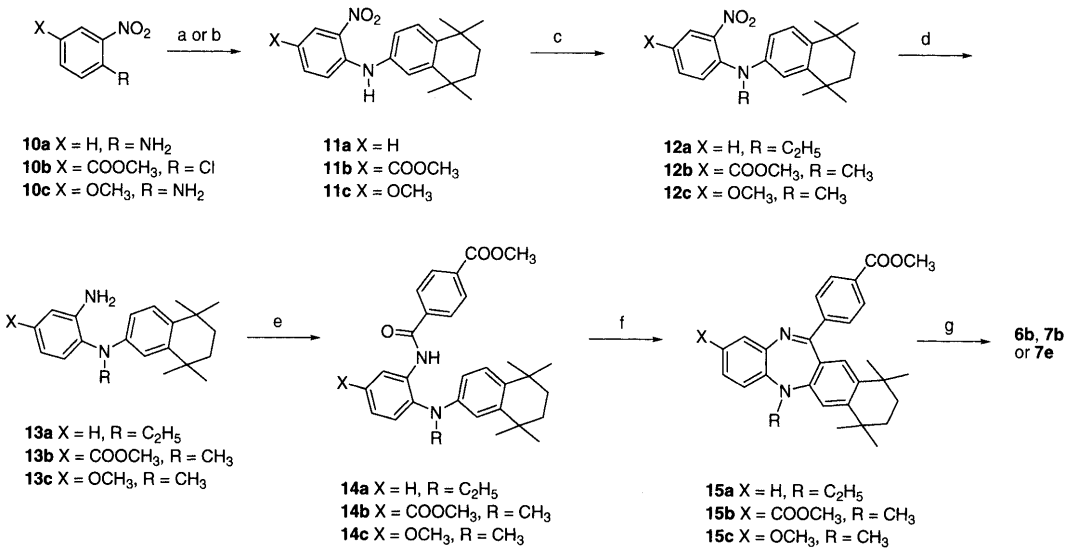
Introduction of various functional groups at the unsubstituted benzo group of HX600 (**6a**) is illustrated in Chart 2. Nitration of **15d** with potassium nitrate (1.6 eq) in sulfuric acid afforded one mononitrated compound **16** (46% with 42% recovery of **15d**), in which the position of the nitro group was determined by an Nuclear Overhauser effect (NOE) experiment. Compound **16** was converted to an acetamido derivative **17** by reduction with Fe under acidic conditions, followed by acetylation. Bromination of **15d** occurred at the same position as in the case of nitration to afford **18** as the sole product (51%). Reaction of **18** with phenylboronic acid in the presence of $[(C_6H_5)_3P]_4Pd$ gave a biphenyl derivative **19** (36%). Compounds **16**—**19** were hydrolyzed under basic conditions to yield the corresponding carboxylic acids, **7a**, **c**, **d** and **g**.

Treatment of **15d** with sodium cyanoborohydride in the presence of trifluoroacetic acid (TFA) gave a reduced product **20** (90%). Both compound **20** and its *N*-methylated analog **21** were hydrolyzed under basic conditions to afford HX710 (**8a**) and HX711 (**8b**), respectively. Other dihydrogenated compounds **8c**—**e** were prepared by similar methods.

Inhibition of Retinoidal Activities in HL-60 Cell Assay

The biological activities of the synthesized compounds were evaluated in terms of the induced differentiation of human promyelocytic leukemia cells HL-60,[13,14] determined by means of nitro blue tetrazolium (NBT) reduction assay.[15] All of the diazepine derivatives **6**—**8** were completely inactive below 1×10^{-6} M in this assay. Their effects on Am80 (**2**)-induced HL-60 cell differentiation are summarized in Table 1, and data for selected compounds are shown in Figs. 3 and 4.

Replacement of the *N*-methyl group of the retinoid synergist HX600 (**6a**) affected the retinoid-regulating activity. Low concentration of HX602 (**6b**), having an *N*-ethyl group, enhanced the activity of 3×10^{-10} M Am80 like HX600 (**6a**), but 3×10^{-7} M HX602 (**6b**) decreased the percentage of differentiated cells to the basal level (Fig. 3). HX603 (**6c**) and



10a X = H, R = NH_2
10b X = $COOCH_3$, R = Cl
10c X = OCH_3, R = NH_2

11a X = H
11b X = $COOCH_3$
11c X = OCH_3

12a X = H, R = C_2H_5
12b X = $COOCH_3$, R = CH_3
12c X = OCH_3, R = CH_3

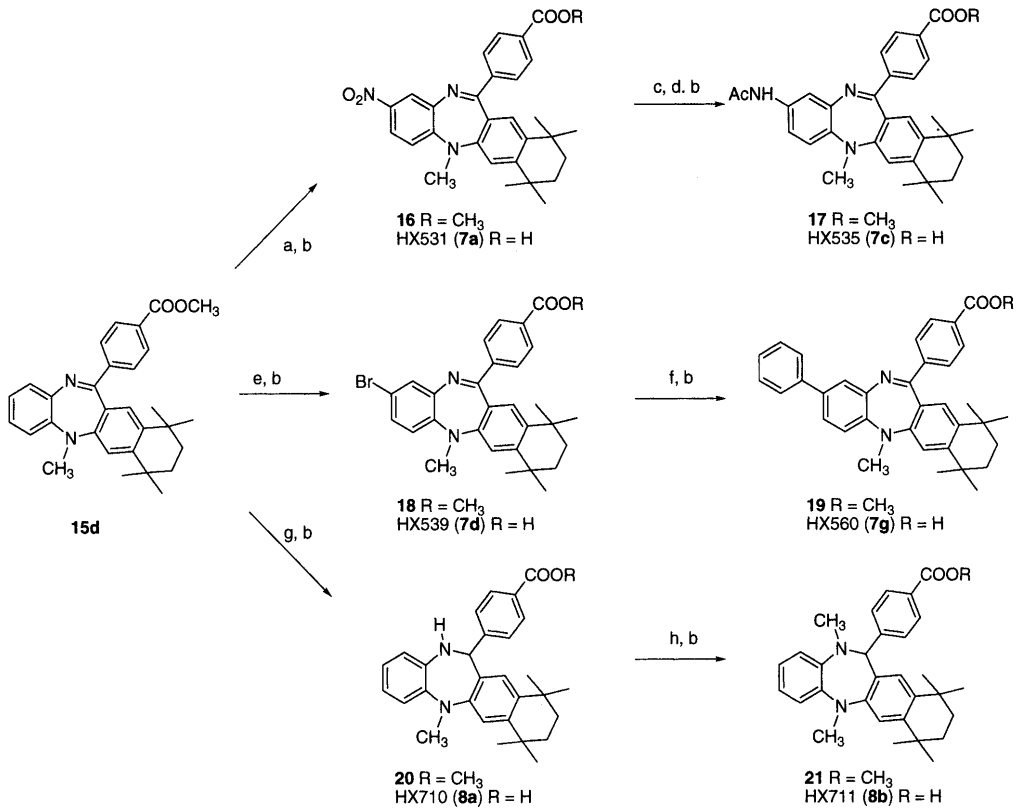
13a X = H, R = C_2H_5
13b X = $COOCH_3$, R = CH_3
13c X = OCH_3, R = CH_3

14a X = H, R = C_2H_5
14b X = $COOCH_3$, R = CH_3
14c X = OCH_3, R = CH_3

15a X = H, R = C_2H_5
15b X = $COOCH_3$, R = CH_3
15c X = OCH_3, R = CH_3

(a) 6-bromo-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene / copper iodide / K_2CO_3 / Δ; (b) 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthylamine / copper iodide / K_2CO_3 / Δ; (c) NaH / DMF; R-I; (d) H_2 / Pd-C; (e) *p*-CH_3OOC-Ph-COCl / pyridine / benzene; (f) PPA; (g) NaOH / EtOH

Chart 1



(a) KNO_3 / H_2SO_4; (b) NaOH / EtOH; (c) Fe / HCl; (d) Ac_2O / pyridine; (e) Br / Fe / CCl_4; (f) $PhB(OH)_2$ / $(Ph_3P)_4Pd$; (g) $NaBH_3CN$ / TFA; (h) NaH / DMF; CH_3I.

Chart 2

Table 1. Effect of Azepine Derivatives on Retinoidal Activity in HL-60 Cell Assay

Compound	Activity	IC_{50}, M[a)]
HX600 (**6a**)	Synergistic	—
HX602 (**6b**)	Antagonistic	2.0×10^{-7}
HX603 (**6c**)	Antagonistic	3.8×10^{-8}
HX604 (**6d**)	Antagonistic	1.6×10^{-7}
HX605 (**6e**)	Inactive	—
HX607 (**6f**)	Inactive	—
HX531 (**7a**)	Antagonistic	1.8×10^{-8}
HX533 (**7b**)	Inactive	—
HX535 (**7c**)	Inactive	—
HX539 (**7d**)	Antagonistic	6.8×10^{-8}
HX541 (**7e**)	Antagonistic	5.3×10^{-7}
HX543 (**7f**)	Inactive	—
HX560 (**7g**)	Antagonistic	5.5×10^{-7}
HX710 (**8a**)	Synergistic	—
HX711 (**8b**)	Antagonistic	1.2×10^{-7}
HX741 (**8c**)	Antagonistic	9.5×10^{-8}
HX743 (**8d**)	Antagonistic	2.7×10^{-7}
HX745 (**8e**)	Antagonistic	8.7×10^{-8}

a) IC_{50} was determined as the concentration of the test compound which reduces by half the percentage of differentiated HL-60 cells induced by 3×10^{-10} M Am80. 'Inactive' means that there was no effect below 1×10^{-6} M test compound.

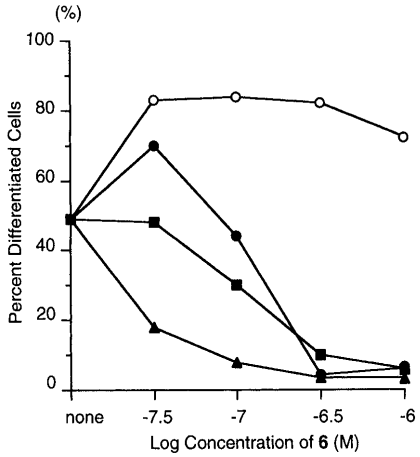


Fig. 3. Effect of Diazepine Derivatives **6a**—**d** on 3×10^{-10} M Am80-Induced HL-60 Cell Differentiation

Added compounds are HX600 (**6a**, ○), HX602 (**6b**, ●), HX603 (**6c**, ▲) and HX604 (**6d**, ■). Vertical scale is percent differentiated cells deduced from NBT reduction assay. The effect of HX600 reached a muximum at 5×10^{-8} M in these experimental conditions.

HX604 (**6d**), with an *n*-propyl and *n*-butyl group, respectively, more strongly inhibited Am80-induced cell differentiation, their IC_{50} values being 3.8×10^{-8} and 1.6×10^{-7} M, respectively. Compounds having a longer *N*-alkyl group, such as HX605 (**6e**) and HX607 (**6f**), did not affect the activity of Am80.

A nitrated analog, HX531 (**7a**), also inhibited the differentiation-inducing activity of Am80 (Fig. 4b). The percentage of differentiated cells (63%) induced by 3×10^{-10} M Am80 was decreased to the basal level by the addition of 1×10^{-7} M HX531 (**7a**), and that (80%) induced by 1×10^{-9} M Am80 (**2**) was decreased to 71%, 20% and 3% by the addition of 1×10^{-7}, 3×10^{-7}, and 1×10^{-6} M HX531 (**7a**), respectively. Similar inhibitory activity was observed in the compounds having a bromo (**7d**), methoxy (**7e**), or phenyl group (**7g**) instead of the nitro group of HX531 (**7a**), although the potency is slightly weaker (Table 1), but the compounds with a car-

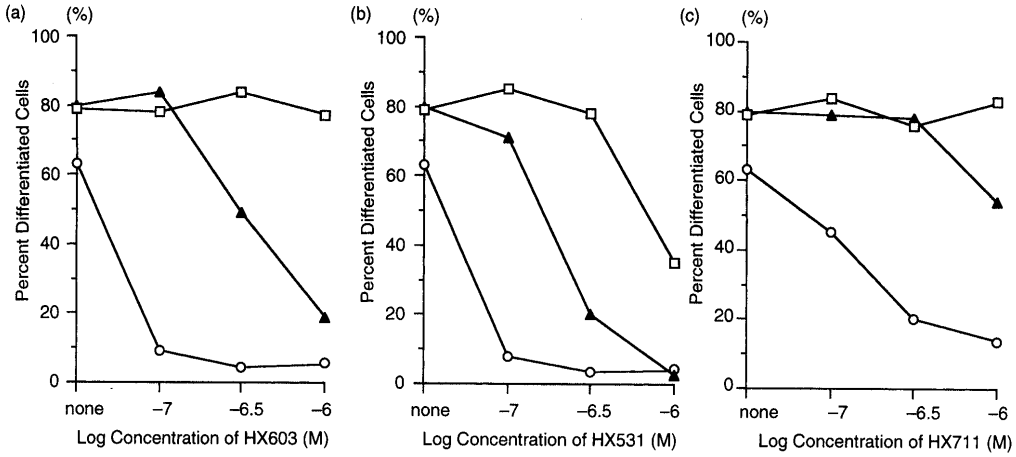


Fig. 4. Inhibition of Am80-induced HL-60 Cell Differentiation by *a*) HX603 (**6c**), *b*) HX531 (**7a**) and *c*) HX711 (**8b**)

Concentrations of Am80 are 3×10^{-9} M (□), 1×10^{-9} M (▲), and 3×10^{-10} M (○). Vertical scale scale is percent differentiated cells deduced from NBT reduction assay.

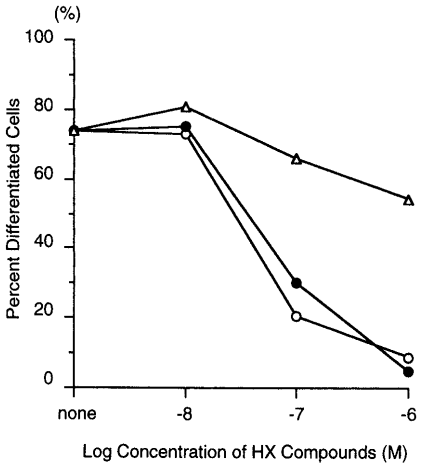


Fig. 5. Inhibition of HL-60 Cell Differentiation Induced by the Comdination of 1×10^{-10} M Am80 and 1×10^{-7} M HX600 (**6a**)

Added compound is HX603 (**6c**, ○), HX531 (**7a**, ●) and HX711 (**8b**, △). Vertical scale is percent differentiated cells deduced from NBT reduction assay.

boxyl (**7b**) or an acetamido group (**7c**) at the same position were inactive below 1×10^{-6} M. In the structure of HX541 (**7e**), the replacement of the *N*-methyl group with an *n*-propyl group caused loss of the activity.

HX710 (**8a**), a dihydrogenated analog of HX600 (**6a**), was rather unstable, and exhibited weak retinoid synergistic activity. *N*-Methylation of the secondary amino group of HX710 (**8a**), yielding HX711 (**8b**), resulted in inhibitory activity towards Am80 (Fig. 4c). Three dihydrogenated analogs of HX541 (**7e**) with different *N*-alkyl groups, **8c**—**e**, also suppressed the activity of Am80, and the length of the *N*-substituent seems to be unimportant, since their IC_{50} values are similar to each other.

The effect of selected antagonistic diazepines, HX603 (**6c**), HX531 (**7a**) and HX711 (**8b**), on HL-60 cell differentiation induced by cotreatment with Am80 and HX600 (**6a**), was next examined. HX600 (**6a**) binds to the RXR site of RAR–RXR heterodimers, and enhances the potency of RARs agonists.[5] The percentage of the differentiated cells (10%) induced by 1×10^{-10} M Am80 was increased to 74% by the addition of 1×10^{-7} M HX600 (**6a**). The synergistic activity of Am80 and HX600 (**6a**) was inhibited dose-dependently by addition of HX603 (**6c**), HX531 (**7a**) or HX711 (**8b**) (Fig. 5). In this respect, HX603 (**6c**) and HX531 (**7a**) were more active than HX711 (**8b**), and completely inhibited the differentiation of HL-60 cells at 1×10^{-6} M. Thus, these antagonistic diazepines seem to inhibit the action of RAR–RXR heterodimers.

Effect on the Activation of Retinoid Receptors by Retinoids Most retinoidal activities can be attributed to the activation of the heterodimeric nuclear receptors, RARs–RXRs. The mechanism of the inhibitory activities of the novel diazepinylbenzoic acids is assumed to be antagonism at the nuclear receptors. Therefore, the effects of the selected diazepine derivatives, HX603 (**6c**), HX531 (**7a**), HX711 (**8b**) and an RAR pan-antagonist LE540 (**4**) for comparison, on the activation of retinoid receptors were investigated by transient transactivation assay (Fig. 6).[6d] None of the diazepine derivatives when examined alone activated any retinoid receptor (data not shown). LE540 (**4**) dose-dependently inhibited the activation of the three RARs induced by 1×10^{-8} M retinoic acid (**1a**), while LE540 (**4**) only at 1×10^{-5} M repressed the RXR activation induced by 1×10^{-8} M LGD1069 (**5**). HX603 (**6c**) potently inhibited activation of the three RXRs. For example, 1×10^{-6} M HX603 (**6c**) decreased the transactivation activity of RXRα, β and γ induced by 1×10^{-8} M LGD1069 (**5**) to 44, 37 and 68%, respectively. The antagonistic activity towards RAR activation of HX603 (**6c**) was weaker. HX531 (**7a**) showed RAR- and RXR-pan-antagonistic activity with similar IC_{50} values, while HX711 (**8b**) inhibited the activation of all six receptors very weakly in this transactivation assay.

Discussion

Recent structural developments in the retinoid area, in the light of the extensive progress in understanding the action mechanisms of retinoids at the specific nuclear receptors, have yielded not only potent synthetic retinoids, but also compounds such as RAR antagonists[3—5] and RXR agonists[5,7,8] which exhibit no retinoidal activity by themselves, but modulate retinoidal action through the nuclear receptors. Previously we reported that the dibenzodiazepine skeleton is a unique structural unit for the construction of retinoid-regulatory compounds, *i. e.*, LE135 (**3**) and HX600 (**6a**), two isomeric diazepines with the same substituent at different positions, are an RAR antagonist and an RXR agonist (synergist

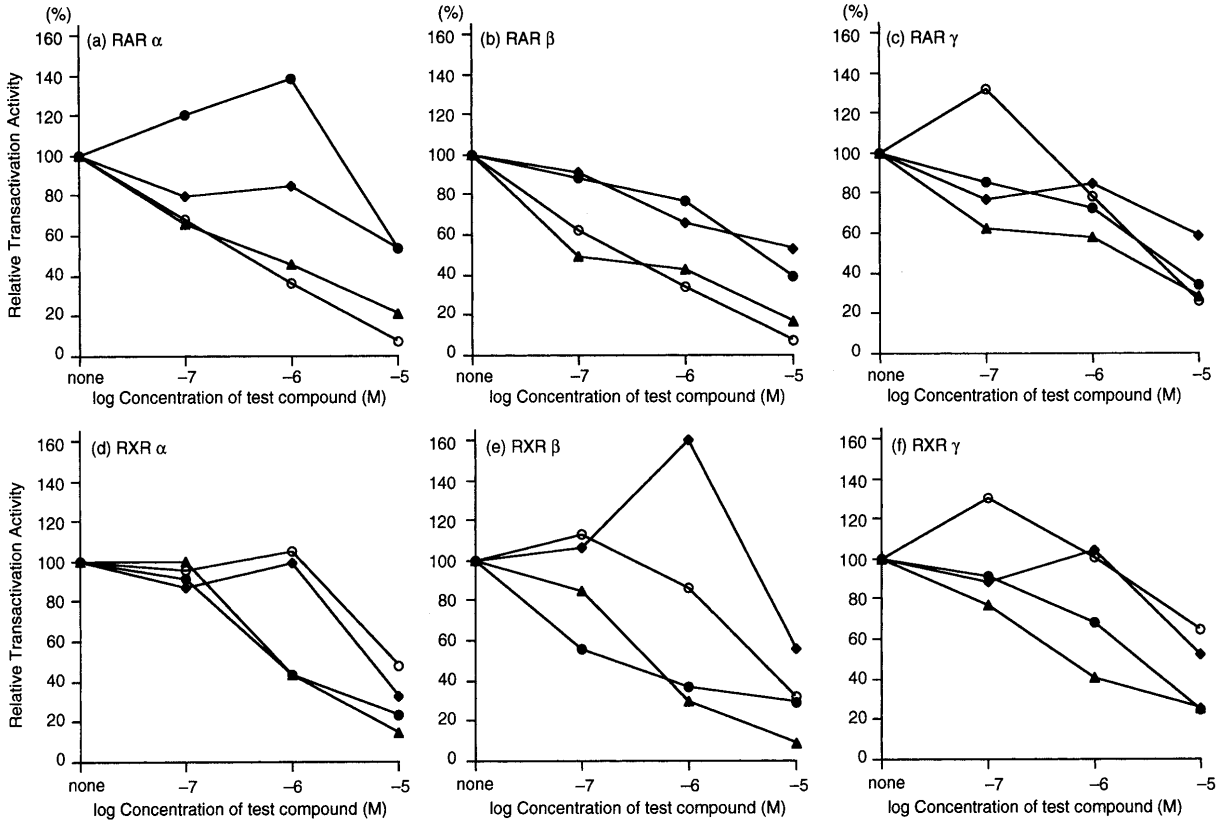


Fig. 6. CAT Assays in Cos-1 Cells Transiently Transfected with RAR/DR5-tk-CAT (RARα, β and γ for a, b and c, Respectively) or RXR/DR1-tk-CAT (RXRα, β and γ for α, e and f, Respectively)

The vertical scale is the 1×10^{-8} M retinoic acid-induced RAR or 1×10^{-8} M LGD1069-induced RXR transactivation activity. The values were normalized to that obtained when retionic acid (**1a**) or LGD1069 (**5**) alone was added, taken as 100. The horizontal scale is the molar concentration of the added compound. Added compounds were LE540 (**4**, ○), HX603 (**6c**, ●), HX531 (**7a**, ▲), and HX711 (**8b**, ◆).

with RAR ligands), respectively.[5] The present results showed that modification of the RXR agonist HX600 (**6a**) afforded RXR antagonists. The retinoid antagonists in this study have three types of structural modifications, (1) elongation of the *N*-methyl group, (2) introduction of a substituent at the benzo group, and (3) dihydrogenation of HX600 (**6a**).

In the case of the HX600 series (**6a**—**f**), moderate size of the *N*-alkyl group, such as *n*-propyl or *n*-butyl, is necessary for the antagonistic activity. When the alkyl group is shorter or longer, the compounds exhibited retinoid synergistic (RXR agonistic) or no activity, respectively. This is different from the structure–activity relationship of the RAR antagonist LE135 (**3**), where the elongation of the *N*-methyl group did not affect the antagonistic activity.[5]

In the structure of HX600 (**6a**), the cyclic alkyl group and the carboxyl group are necessary for the activity, and the unsubstituted benzo group is important for the potency. Thus, the replacement of the anilino moiety with an acetamido group decreased the synergistic activity.[5] The introduction of a nitro group into HX600 (**6a**) changed the synergistic activity into antagonistic activity. The potency of the nitro derivative HX531 (**7a**) is the highest among the antagonistic diazepines examined. However, the role of the nitro group is obscure, since the nitro group can be replaced by an electron-donating methoxy group or a hydrophobic phenyl group without significant loss of the activity.

The dihydrogenated derivatives (**8**) may have different conformations from the parent azepine compounds, and are retinoid antagonists in HL-60 cell assay, except **8a** (having a secondary amino moiety). The methoxy group of HX541 (**7e**) is significant for the activity, but the introduction of a methoxyl group into HX711 (**8b**) did not increase the antagonistic potency.

The transactivation assay suggested that the inhibition of retinoidal activities by the diazepine derivatives **6**—**8** is mediated by retinoid nuclear receptors. The activations of transiently transfected RARs induced by retinoic acid (**1a**) were dose-dependently suppressed by LE540 (**4**) and HX531 (**7a**), and HX603 (**6c**) and HX711 (**8b**) were very weak inhibitors. On the other hand, the activations of RXRs induced by LGD1069 (**5**) were suppressed by HX531 (**7a**) and HX603 (**6c**). Thus, HX603 (**6c**) and HX531 (**7a**) are RXR pan-antagonists. In the transient transactivation assay, HX603 (**6c**) is the most selective RXR antagonist. LE540 (**4**) and HX711 (**8b**) were active only at 1×10^{-5} M.

LG100754 (**9**) is a known RXR antagonist, but this compound is RXR homodimer-specific and acts as an agonist towards RAR–RXR heterodimers, rather than an antagonist.[12] Thus, LG100754 (**9**) induced the differentiation of the acute promyelocytic leukemia cell line NB4, like retinoids (RAR agonists). Considering the inhibitory activity of the diazepines **6**—**8** in HL-60 cell assay, they should antagonize the activation of RAR–RXR heterodimers, since the heterodimers have a significant role in retinoid-induced HL-60 cell differentiation. Indeed, HX603 (**6c**), LE540 (**4**) and HX711 (**8b**) inhibited the HL-60 cell differentiation not only by retinoid (RAR agonist) alone, but also by the combination of a retinoid with an RXR agonist (retinoid synergist). We

confirmed the antagonistic activity of HX531 (**7a**) and HX711 (**8b**) on the activation of RAR–RXR heterodimer induced by 9-*cis*-retinoic acid (**1b**) using Hela cells transiently transfected with both RARα and RXRα (data not shown).

At present, it is most reasonable to conclude that the RXR antagonists described here inhibited the actions of both RXR homodimers and RAR–RXR heterodimers. Interestingly, HX531 (**7a**) and HX711 (**8b**) inhibited the activity of a constitutively active RXR mutant.[11] Further, HX531 (**7a**) suppressed the activity of heterodimers of the constitutively active RXR mutant with RAR or orphan receptor NGFI-B. These results indicated that HX531 (**7a**) and the related RXR antagonists modulate not only retinoidal actions, but also the activities of heterodimers of RXR with other nuclear receptors.

In conclusion, we have developed novel RXR-antagonistic diazepinylbenzoic acids. Some of them, such as HX531 (**7a**), antagonized RXRs besides RARs. Among the synthesized compounds, HX603 (**6c**) is most RXR-selective antagonist. Although their stereochemical relationship with RAR–RXR heterodimers remains to be established, this result implies an allosteric effect of the HX603-bound RXR onto its Am80-liganded RAR partner. The novel RXRs antagonists described here will be useful tools for the regulation of the pleiotropic activities of retinoids at the nuclear receptor level, and for elucidation of the regulation mechanisms of gene networks by various nuclear receptors.

Experimental

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.

4-(5*H*-5-Ethyl-2,3-(2,5-dimethyl-2,5-hexano)dibenzo[*b*,*e*][1,4]diazepin-11-yl)benzoic Acid (HX602, 6b) A mixture of 6-bromo-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (30.0 g, 0.11 mol), *o*-nitroaniline (**10a**, 56.1 g, 0.41 mol), potassium carbonate (56.1 g, 0.41 mol) and copper iodide (4.53 g) in *o*-xylene (300 ml) was heated at 150 °C for 14 h. After removal of the solvent, the residue was purified by silica gel column chromatography (AcOEt : *n*-hexane=1 : 100) to give **11a** (30.0 g, 82%). **11a**: red plates (*n*-hexane); mp 118 °C; ^{1}H-NMR ($CDCl_3$) δ 9.49 (s, 1H), 8.20 (dd, 1H, J=8.8, 1.5 Hz), 7.35 (s, 1H), 7.33 (s, 1H), 7.21 (dd, 1H, J=8.6, 1.5 Hz), 7.18 (d, 1H, J=2.2 Hz), 7.05 (dd, 1H, J=8.4, 2.2 Hz), 6.73 (m, 1H), 1.71 (s, 4H), 1.30 (s, 6H), 1.29 (s, 6H).

A solution of **11a** (500 mg, 1.54 mmol) in dimethylformamide (DMF, 10 ml) was added to a suspension of NaH (60%, 92 mg, 2.31 mmol) in DMF (1 ml), and the mixture was stirred for 30 min. Then, ethyl iodide (0.25 ml) was added, and the whole was stirred for 1 h. After removal of the solvent, 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 $MgSO_4$. Removal of the solvent under vacuum gave crude **12a** (543 mg, quant.). **12a**: ^{1}H-NMR ($CDCl_3$) δ 7.77 (dd, 1H, J=8.0, 1.8 Hz), 7.53 (dt, 1H, J=8.4, 1.8 Hz), 7.33 (dd, 1H, J=8.0, 1.1 Hz), 7.19 (dt, 1H, J=8.4, 1.5 Hz), 7.12 (s, 1H), 7.10 (s, 1 H), 6.60 (d, 1H, J=2.9 Hz), 6.55 (dd, 1H, J=8.4, 2.6 Hz), 3.75 (q, 2H, J=7.0, 1.1 Hz), 1.63 (s, 4H), 1.25 (t, 3H, J=7.0 Hz), 1.22 (s, 6H), 1.17 (s, 6H).

12a (540 mg, 1.53 mmol) was dissolved in 20 ml of ethanol, and hydrogenated over 10% Pd–C (55 mg) for 1 h. After filtration and removal of the solvent, the residue was chromatographed on silica gel (AcOEt : *n*-hexane=1 : 8) to give **13a** (423 mg, 86%). **13a**: ^{1}H-NMR ($CDCl_3$) δ 7.07 (m, 3H), 6.82 (dd, 1H, J=7.8, 1.2 Hz), 6.76 (dt, 1H, J=7.8, 1.5 Hz), 6.58 (d, 1H, J=2.9 Hz), 6.39 (dd, 1H, J=8.8, 2.9 Hz), 3.61 (q, 2H, J=7.0 Hz), 1.64 (s, 4H), 1.23 (s, 6H), 1.22 (s, 6H), 1.21 (t, 3H, J=5.3 Hz).

Terephthalic acid monomethyl ester chloride (381 mg, 1.91 mmol) was added to a solution of **13a** (420 mg, 1.30 mmol) in dry benzene (10 ml) and pyridine (2 ml). The mixture was stirred for 4 h, then poured into 2 N hydrochloric acid, and extracted with AcOEt. The organic layer was dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (AcOEt : *n*-hexane=1 : 8) to give **14a** (631 mg, quant.). **14a**: ^{1}H-NMR ($CDCl_3$) δ 8.59 (dd, 1H, J=8.4, 1.5 Hz), 8.47 (s, 1H), 7.99 (d, 2 H, J=8.4 Hz), 7.46 (d, 2H, J=8.4 Hz), 7.33 (dt, 1H, J=8.4, 1.5 Hz), 7.26 (dd, 1H, J=8.4, 1.5 Hz), 7.20 (dt, 1 H, J=7.3, 1.5 Hz), 7.17 (d, 1H, J=8.4 Hz), 6.65 (d, 1H, J=2.6 Hz), 6.54 (dd, 1H, J=8.8, 2.6 Hz), 3.93 (s, 3H), 3.74 (q, 2 H, J=7.0 Hz), 1.64 (s, 4H), 1.23 (s, 6H), 1.21 (t, 3 H, J=7.0 Hz), 1.15 (s, 6H).

A solution of **14a** (630 mg, 1.30 mmol) in a small amount of CH_2Cl_2 was added to PPA (6.0 g), and the mixture was heated at 110 °C for 18 h. After cooling, water was added, and the whole was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (AcOEt : *n*-hexane=1 : 6) to afford **15a** (104 mg, 17%). **15a**: ^{1}H-NMR ($CDCl_3$) δ 8.08 (d, 2H, J=8.4 Hz), 7.89 (d, 2H, J=8.4 Hz), 7.32 (d, 1H, J=6.6 Hz), 7.15 (dt, 1H, J=7.7, 1.8 Hz), 7.09 (dt, 1H, J=7.7, 1.5 Hz), 6.96 (dd, 1H, J=6.6, 1.5 Hz), 6.90 (s, 1H), 6.88 (s, 1H), 3.95 (s, 3H), 3.71 (m, 2H), 1.68 (m, 4H), 1.31 (s, 3H), 1.25 (s, 3H), 1.24 (t, 3H, J=7.0 Hz), 1.12 (s, 3H), 1.03 (s, 3H).

A solution of **15a** (53 mg, 0.11 mmol) in ethanol (4 ml) and 2 N NaOH (2 ml) was stirred at room temperature for 2 h. The mixture was poured into 2 N hydrochloric acid, and extracted with CH_2Cl_2. The organic layer was washed with brine, and dried over $MgSO_4$. After evaporation, the crude product was purified by silica gel column chromatography (CH_2Cl_2 : CH_3OH=20 : 1, then 8 : 1) to give HX602 (**6b**) as a colorless oil (33 mg, 64%). HX602 (**6b**): ^{1}H-NMR ($CDCl_3$) δ 8.15 (d, 2H, J=8.0 Hz), 7.92 (d, 2H, J=7.7 Hz), 7.32 (d, 1H, J=8.1 Hz), 7.15 (t, 1H, J=6.7 Hz), 7.08 (t, 1H, J=7.5 Hz), 6.96 (d, 1H, J=8.0 Hz), 6.90 (s, 1H), 6.89 (s, 1H), 3.69 (m, 2H), 1.65 (m, 4H), 1.31 (s, 3H), 1.25 (s, 3H), 1.13 (s, 3H), 1.04 (s, 3H), 0.86 (t, 3H, J=8.0 Hz).

Other diazepine derivatives (**6c—6f**) were synthesized from **11a** and the corresponding *n*-alkyl halide according to the above method.

HX603 (**6c**): Yellow prisms (EtOH–H_2O); mp 248.5 °C; ^{1}H-NMR ($CDCl_3$) δ 8.16 (d, 2H, J=8.4 Hz), 7.92 (d, 2H, J=8.4 Hz), 7.33 (d, 1H, J=7.7 Hz), 7.16 (t, 1H, J=6.4 Hz), 7.09 (t, 1H, J=6.4 Hz), 6.98 (d, 1H, J=7.7 Hz), 6.92 (s, 1H), 6.87 (s, 1H), 3.69 (m, 1H), 3.56 (m, 1H), 1.65 (m, 6H), 1.32 (s, 3H), 1.26 (s, 3H), 1.14 (s, 3H), 1.05 (s, 3H), 0.93 (t, 3H, J=7.3 Hz); *Anal.* Calcd for $C_{31}H_{34}N_2O_2$: C, 79.67; H, 7.18; N, 5.95. Found: C, 79.79; H, 7.34; N, 6.05.

HX604 (**6d**): Orange prisms (EtOH–H_2O); mp 253 °C; ^{1}H-NMR ($CDCl_3$) δ 8.15 (d, 2H, J=8.8 Hz), 7.91 (d, 2H, J=8.4 Hz), 7.33 (br s, 1H), 7.16 (t, 1H, J=6.5 Hz), 7.09 (t, 1H, J=6.2 Hz), 6.98 (d, 1H, J=8.3 Hz), 6.91 (s, 1H), 6.87 (s, 1H), 3.67 (m, 2H), 1.65 (m, 6H), 1.40 (m, 2H), 1.32 (s, 3H), 1.26 (s, 3H), 1.14 (s, 3H), 1.04 (s, 3H), 0.86 (t, 3H, J=7.3 Hz); *Anal.* Calcd for $C_{32}H_{36}N_2O_2$: C, 79.97; H, 7.55; N, 5.83. Found: C, 79.74; H, 7.70; N, 5.62.

HX605 (**6e**): Red prisms (EtOH–H_2O); mp 220 °C; ^{1}H-NMR ($CDCl_3$) δ 8.15 (d, 2H, J=8.4 Hz), 7.91 (d, 2H, J=8.4 Hz), 7.33 (br s, 1H), 7.16 (t, 1H, J=7.0 Hz), 7.07 (t, 1H, J=6.8 Hz), 6.98 (d, 1H, J=8.1 Hz), 6.91 (s, 1H), 6.87 (s, 1H), 3.65 (m, 2H), 1.65 (m, 6H), 1.32 (m, 2H), 1.31 (s, 3H), 1.26 (s, 3H), 1.13 (s, 3H), 1.04 (s, 3H), 0.79 (t, 3H, J=7.0 Hz); *Anal.* Calcd for $C_{33}H_{38}N_2O_2$: C, 80.13; H, 7.44; N, 5.66. Found: C, 79.85; H, 7.69; N, 5.36.

HX607 (**6f**): Orange needles (EtOH–H_2O); mp 225 °C; ^{1}H-NMR ($CDCl_3$) δ 8.15 (d, 2H, J=8.1 Hz), 7.92 (d, 2H, J=8.1 Hz), 7.33 (d, 1H, J=7.7 Hz), 7.16 (t, 1H, J=7.3 Hz), 7.09 (t, 1H, J=7.3 Hz), 6.98 (d, 1H, J=8.1 Hz), 6.91 (s, 1H), 6.87 (s, 1H), 3.69 (m, 1H), 3.57 (m, 1H), 1.62 (m, 6H), 1.31 (s, 3H), 1.26 (s, 3H), 1.13 (s, 3H), 1.04 (s, 3H), 1.42—1.08 (br m, 8H), 0.79 (t, 3H, J=6.8 Hz); *Anal.* Calcd for $C_{35}H_{42}N_2O_2$: C, 80.45; H, 8.19; N, 5.16. Found: C, 80.42; H, 8.10; N, 5.36.

4-(5*H*-2,3-(2,5-Dimethyl-2,5-hexano)-5-methyl-8-nitrodibenzo-[*b*,*e*][1,4]diazepin-11-yl)benzoic Acid (HX531, 7a) KNO_3 (73 mg, 0.72 mmol) was added to a solution of **15d** (200 mg, 0.44 mmol) in sulfuric acid (12 ml) at 0 °C. After 2.5 h, the mixture was 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 $MgSO_4$. After evaporation, the residue was purified by silica gel column chromatography (AcOEt : *n*-hexane=1 : 8) to give methyl 4-(5*H*-2,3-(2,5-dimethyl-2,5-hexano)-5-methyl-8-nitrodibenzo[*b*,*e*][1,4]diazepin-11-yl)benzoate (**16**, 100 mg, 45.5%) and recovered **15d** (84 mg). **16**: ^{1}H-NMR ($CDCl_3$) δ 8.14 (d, 1H, J=2.9 Hz), 8.10 (d, 2H, J=8.4 Hz), 8.02 (dd, 1H, J=8.8, 2.9 Hz), 7.88 (d, 2H, J=8.8 Hz), 7.00 (d, 1H, J=9.1 Hz), 6.93 (s, 1H), 6.90 (s, 1H), 3.97 (s, 3H), 3.32 (s, 3H), 1.69 (m, 4H), 1.32 (s, 3H), 1.28 (s, 3H), 1.14 (s, 3H), 1.07 (s, 3H).

Compound **16** was hydrolyzed under basic conditions (2 N NaOH/EtOH) to give HX531 (**7a**, 89%). HX531 (**7a**): Yellow needles (EtOH–H_2O); mp>300 °C; ^{1}H-NMR ($CDCl_3$) δ 8.15 (m, 3H,), 8.01 (dd, 1H, J=8.8, 2.6 Hz), 7.90 (d, 2H, J=7.3 Hz), 7.00 (d, 1H, J=9.2 Hz), 6.93 (s, 1H), 6.92 (s,

1H), 3.31 (s, 3H), 1.65 (m, 4H), 1.32 (s, 3H), 1.27 (s, 3H), 1.14 (s, 3H), 1.07 (s, 3H); *Anal.* Calcd for $C_{29}H_{29}N_3O_4$: C, 72.03; H, 6.04; N, 8.69. Found: C, 71.89; H, 6.25; N, 8.54.

4-(5*H*-8-Carboxy-2,3-(2,5-dimethyl-2,5-hexano)-5-methyldibenzo-[*b*,*e*][1,4]diazepin-11-yl)benzoic Acid (HX533, 7b) A mixture of methyl 4-chloro-3-nitrobenzoate (**10b**, 319 mg, 1.48 mmol), 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthylamine (450 mg, 2.22 mmol), potassium carbonate (204 mg, 1.48 mmol) and copper iodide (13 mg) in *o*-xylene (20 ml) was heated at 150 °C for 15 h. After removal of the solvent, the residue was purified by silica gel column chromatography (AcOEt : *n*-hexane = 1 : 4) to give **11b** (395.5 mg, 70%). **11b**: Orange powder (EtOH–H_2O); mp 81.5 °C; ^{1}H-NMR ($CDCl_3$) δ 9.78 (s, 1H), 8.92 (d, 1H, J=1.8 Hz), 7.95 (dd, 1H, J=8.8, 1.8 Hz), 7.37 (d, 1H, J=8.4 Hz), 7.18 (d, 1H, J=2.2 Hz), 7.17 (d, 1H, J=9.2 Hz), 7.04 (dd, 1 H, J=8.4, 2.2 Hz), 3.91 (s , 3H), 1.71 (s, 4H), 1.30 (s, 6H), 1.28 (s, 6H).

A solution of **11b** (380 mg, 0.99 mmol) in DMF (13 ml) was added to a suspension of NaH (60%, 60 mg, 1.49 mmol) in DMF (1 ml), and the mixture was stirred for 30 min. Then, methyl iodide (0.13 ml) was added, and stirring was continued for 1 h. After removal of the solvent, 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 $MgSO_4$. Removal of the solvent under vacuum gave crude **12b** (375 mg, 95%). **12b**: ^{1}H-NMR ($CDCl_3$) δ 8.38 (d, 1H, J=2.2 Hz), 8.05 (dd, 1H, J=8.8, 2.2 Hz), 7.24 (d, 1H, J=8.4 Hz), 7.19 (d, 1H, J=8.8 Hz), 6.87 (d , 1H, J=2.6 Hz), 6.84 (dd , 1H, J=8.4, 2.6 Hz), 3.92 (s, 3H), 3.37 (s, 3H), 1.65 (s, 4H), 1.25 (s, 6H), 1.19 (s, 6H).

12b (360 mg, 0.91 mmol) was hydrogenated over 10% Pd–C (40 mg) in 20 ml of ethanol at 50 °C for 2.5 h. After filtration, the solvent was removed under vacuum to give **13b** (307 mg, 92%). **13b**: ^{1}H-NMR ($CDCl_3$) δ 7.48 (d, 1H, J=1.8 Hz), 7.43 (dd, 1H, J=8.4, 2.2 Hz), 7.13 (d, 1H, J=8.8 Hz), 7.10 (d, 1H, J=8.1 Hz), 6.63 (d, 1H, J=2.6 Hz), 6.51 (dd, 1H, J=8.8, 2.6 Hz), 3.90 (s, 3H), 3.21 (s, 3H), 1.65 (s, 4H), 1.24 (s, 6H), 1.21 (s, 6H).

Terephthalic acid monomethyl ester chloride (78 mg, 0.39 mmol) was added to a solution of **13b** (130 mg, 0.36 mmol) in dry benzene (10 ml) and pyridine (1 ml), and the mixture was stirred for 3 h. The mixture was poured into 2 N hydrochloric acid, and extracted with AcOEt. The organic layer was dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (CH_2Cl_2) to give **14b** (164 mg, 87%). **14b**: ^{1}H-NMR ($CDCl_3$) δ 9.13 (d, 1H, J=2.2 Hz), 8.13 (s, 1H), 7.97 (d, 2H, J=8.8 Hz), 7.92 (dd, 1H, J=8.4, 1.8 Hz), 7.34 (d, 2H, J=8.8 Hz), 7.31 (d, 1H, J=8.5 Hz), 7.23 (d, 1H, J=8.4 Hz), 6.71 (d, 1H, J=2.2 Hz), 6.69 (dd, 1H, J=8.4, 2.9 Hz), 3.95 (s, 3H), 3.93 (s, 3H), 3.38 (s, 3H), 1.63 (s, 4H), 1.23 (s, 6H), 1.12 (s, 6H).

A solution of **14b** (160 mg, 0.30 mmol) in a small amount of CH_2Cl_2 was added to PPA (4.0 g), and the mixture was heated at 110 °C for 2 h. After cooling, water was added and the whole was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (AcOEt : *n*-hexane = 1 : 3) to afford **15b** (41 mg, 26%). **15b**: Orange prisms (EtOH–H_2O); mp 240 °C; ^{1}H-NMR ($CDCl_3$) δ 8.09 (d, 2H, J=8.4 Hz), 7.99 (s, 1H), 7.89 (d, 2H, J=8.4 Hz), 7.83 (d, 1H, J=8.4 Hz), 6.98 (d, 1H, J=8.4 Hz), 6.93 (s, 1H), 6.88 (s, 1H), 3.96 (s, 3H), 3.90 (s, 3H), 3.29 (s, 3H), 1.65 (m, 4H), 1.32 (s, 3H), 1.27 (s, 3H), 1.12 (s, 3H), 1.05 (s, 3H); *Anal.* Calcd for $C_{32}H_{34}N_2O_4$: C, 75.24; H, 6.71; N, 5.48. Found: C, 75.22; H, 6.83; N, 5.28.

A suspension of **15b** (28 mg, 0.056 mmol) in ethanol (6 ml) and 2 N NaOH (3 ml) was heated at reflux for 2 h. The mixture was poured into 2 N hydrochloric acid, and extracted with CH_2Cl_2. The organic layer was washed with water and brine, then dried over $MgSO_4$. After evaporation, the crude product was purified by recrystallization to give HX533 (**7b**, 23 mg, 88%). **HX533** (**7b**): Orange powder (EtOH–H_2O); mp>300 °C; ^{1}H-NMR (DMSO-d_6) δ 8.03 (d, 2H, J=8.4 Hz), 7.81 (d, 2H, J=8.4 Hz), 7.75 (m, 2H), 7.15 (d, 1H, J=8.8 Hz), 7.08 (s, 1H), 6.88 (s, 1H), 3.26 (s, 3H), 1.62 (m, 4H), 1.30 (s, 3H), 1.26 (s, 3H), 1.11 (s, 3H), 1.02 (s, 3H); HR-MS Calcd for $C_{30}H_{30}N_2O_4$, 482.2206; Found 482.2205.

4-(5*H*-8-Acetamido-2,3-(2,5-dimethyl-2,5-hexano)-5-methyldibenzo-[*b*,*e*][1,4]diazepin-11-yl)benzoic Acid (HX535, 7c) Hydrochloric acid (3.8 ml) and Fe powder (127 mg) were added to a suspension of **16** (120 mg, 0.24 mmol), ethanol (10 ml), and water (5 ml), and the mixture was heated at reflux for 2 h. After filtration, the reaction mixture was made basic by adding NaOH, and extracted with AcOEt. The organic layer was washed with water and brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (AcOEt : *n*-hexane 1 : 8) to give methyl 4-(5*H*-8-amino-2,3-(2,5-dimethyl-2,5-hexano)-5-methyldibenzo-[*b*,*e*][1,4]diazepin-11-yl)benzoate (quant.).

Pyridine (a few drops) was added to a solution of methyl 4-(5*H*-8-amino-2,3-(2,5-dimethyl-2,5-hexano)-5-methyldibenzo[*b*,*e*][1,4]diazepin-11-yl)benzoate (117 mg, 0.25 mmol) in acetic anhydride (5 ml), and the mixture was stirred for 2 h. After removal of excess acetic anhydride, the residue was taken up with water and CH_2Cl_2. The organic layer was washed successively with water, 1 N $NaHCO_3$, 2 N hydrochloric acid, and water, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2 : methanol 10 : 1 then 6 : 1) to give **17** (52 mg), which was hydrolyzed under basic conditions to afford HX535 (**7c**, 57%). HX535 (**7c**): Yellow powder (EtOH–H_2O); mp 240 °C; ^{1}H-NMR (DMSO-d_6) δ 9.83 (s, 1H), 8.02 (d, 2H, J=8.4 Hz), 7.77 (d, 2H, J=8.4 Hz), 7.52 (d, 1H, J=2.6 Hz), 7.31 (dd, 1H, J=8.8, 2.6 Hz), 7.03 (s, 1H), 6.99 (d, 1H, J=8.8 Hz), 6.86 (s, 1H), 3.17 (s, 3H), 2.02 (s, 3H), 1.68 (m, 4H), 1.30 (s, 3H), 1.26 (s, 3H), 1.11 (s, 3H), 1.02 (s, 3H); HR-MS Calcd for $C_{31}H_{33}N_3O_3$, 495.2522; Found 495.2520.

4-(5*H*-8-Bromo-2,3-(2,5-dimethyl-2,5-hexano)-5-methyldibenzo-[*b*,*e*][1,4]diazepin-11-yl)benzoic Acid (HX539, 7d) Iron powder (6 mg) was added to a solution of **15d** (400 mg, 0.88 mmol) in carbon tetrachloride (14 ml), and the mixture was stirred vigorously at 70—80 °C. Bromine (121 mg, 1.04 mmol) was added, and the whole was stirred for 3 h, then poured into 10% sodium bisulfite, and filtrated. The filtrate was extracted with CH_2Cl_2. The organic layer was washed with 2 N NaOH and water, and dried over $MgSO_4$. After evaporation, the residue was purified by silica gel column chromatography (CH_2Cl_2) to give **18** (237 mg, 51%) with recovered **15d** (180 mg). **18**: Orange prisms (EtOH–H_2O); mp 178 °C; ^{1}H-NMR ($CDCl_3$) δ 8.08 (d, 2H, J=8.4 Hz), 7.86 (d, 2H, J=8.4 Hz), 7.46 (d, 1H, J=2.2 Hz), 7.24 (dd, 1H, J=8.4, 2.2 Hz), 6.91 (s, 1H), 6.87 (s, 1H), 6.82 (d, 1H, J=8.4 Hz), 3.96 (s, 3H), 3.23 (s, 3H), 1.64 (m, 4H), 1.31 (s, 3H), 1.27 (s, 3H), 1.12 (s, 3H), 1.05 (s, 3H); *Anal.* Calcd for $C_{30}H_{31}BrN_2O_2$: C, 67.80; H, 5.88; N, 5.27. Found: C, 67.85; H, 5.81; N, 5.37.

Compound **18** was hydrolyzed under basic conditions (2 N NaOH/EtOH) to give HX539 (**7d**, 99%). HX539 (**7d**): Orange prisms (EtOH–H_2O); mp>300 °C; ^{1}H-NMR ($CDCl_3$) δ 8.14 (d, 2H, J=8.4 Hz), 7.89 (d, 2H, J=8.4 Hz), 7.46 (d, 1H, J=2.2 Hz), 7.24 (dd, 1H, J=8.8, 2.5 Hz), 6.92 (s, 1H), 6.88 (s, 1H), 6.83 (d, 1H, J=8.4 Hz), 3.23 (s, 3H), 1.63 (m, 4H), 1.32 (s, 3H), 1.27 (s, 3H), 1.14 (s, 3H), 1.06 (s, 3H); *Anal.* Calcd for $C_{29}H_{29}BrN_2O_2$: C, 67.31; H, 5.65; N, 5.41. Found: C, 67.02; H, 5.71; N, 5.26.

4-(5*H*-2,3-(2,5-Dimethyl-2,5-hexano)-8-methoxy-5-methyldibenzo-[*b*,*e*][1,4]diazepin-11-yl)benzoic Acid (HX541, 7e) A mixture of 4-methoxy-2-nitroaniline (**10c**, 343 mg, 2.04 mmol), 6-bromo-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (600 mg, 2.25 mmol), potassium carbonate (281 mg, 2.04 mmol) and copper iodide (22 mg) in *o*-xylene (20 ml) was heated at 150 °C for 15.5 h. After removal of the solvent, the residue was purified by silica gel column chromatography (*n*-hexane then AcOEt : *n*-hexane = 1 : 8) to give **11c** (629 mg, 87%). **11c**: ^{1}H-NMR ($CDCl_3$) δ 7.62 (d, 1H, J=2.9 Hz), 7.31 (d, 1H, J=8.4 Hz), 7.21 (d, 1H, J=8.5 Hz), 7.14 (d, 1H, J=2.6 Hz), 7.06 (dd, 1H, J=8.5, 2.9 Hz), 7.02 (dd, 1H, J=8.4, 2.6 Hz), 3.82 (s , 3H), 1.67 (s, 4H), 1.26 (s, 6H), 1.25 (s, 6H).

A solution of **11c** (600 mg, 1.69 mmol) in DMF (20 ml) was added to a suspension of NaH (60%, 102 mg, 2.54 mmol) in DMF (1 ml), and the mixture was stirred for 30 min. Then, methyl iodide (0.22 ml) was added, and stirring was continued for 1.5 h. After removal of the solvent, the residue was taken up in water, and extracted with ether. The organic layer was washed with water and brine, and dried over $MgSO_4$. Removal of the solvent under vacuum gave crude **12c** (623 mg, quant.). **12c**: ^{1}H-NMR ($CDCl_3$) δ 7.36 (d, 1H, J=2.9 Hz), 7.28 (d, 1H, J=9.2 Hz), 7.13 (dd, 1H, J=8.8, 2.9 Hz), 7.09 (d, 1H, J=8.8 Hz), 6.52 (d , 1H, J=2.6 Hz), 6.43 (dd , 1H, J=8.4, 2.6 Hz), 3.88 (s, 3H), 3.23 (s, 3H), 1.63 (s, 4H), 1.22 (s, 6H), 1.18 (s, 6H).

12c (620 mg, 1.68 mmol) was hydrogenated over 10% Pd–C (62 mg) in 20 ml of ethanol for 3 h. After filtration, and removal of the solvent, the residue was purified by silica gel column chromatography (AcOEt : *n*-hexane = 1 : 6) to give **13c** (394 mg, 69%). **13c**: ^{1}H-NMR ($CDCl_3$) δ 7.10 (d, 1H, J=8.4 Hz), 6.95 (d, 1H, J=8.4 Hz), 6.60 (d, 1H, J=2.6 Hz), 6.43 (m, 2H), 6.34 (dd, 1H, J=8.8, 2.9 Hz), 3.79 (s, 3H), 3.16 (s, 3H), 1.65 (s, 4H), 1.23 (s, 12H).

Terephthalic acid monomethyl ester chloride (247 mg, 1.27 mmol) was added to a solution of **13c** (390 mg, 1.15 mmol) in dry benzene (20 ml) and pyridine (4 ml), and the mixture was stirred for 1 h, then poured into 2 N hydrochloric acid, and extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (CH_2Cl_2) to give **14c** (483 mg, 84%). **14c**: ^{1}H-NMR ($CDCl_3$) δ 8.60 (s, 1H), 8.30 (d, 1H, J=2.9 Hz), 8.00 (d, 2H, J=8.8 Hz), 7.51 (d, 2H, J=8.8 Hz), 7.19 (d, 1H, J=8.8 Hz), 7.11 (d, 1H, J=8.4 Hz), 6.73 (dd, 1H, J=8.8, 2.9 Hz), 6.65 (d, 1H, J=2.6 Hz), 6.55 (dd, 1H, J=8.8, 2.9 Hz), 3.93 (s, 3H), 3.89 (s, 3H), 3.25 (s, 3H), 1.64 (s, 4H), 1.24 (s, 6H), 1.17 (s, 6H).

A solution of **14c** (480 mg, 0.96 mmol) in a small amount of CH_2Cl_2 was

added to PPA (6.0 g), and the mixture was heated at 110 °C for 6 h. After cooling, water was added, and the whole was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The crude product (389 mg, 84%) was purified by recrystallization from EtOH–H_2O to afford **15c**. **15c**: Orange needles (EtOH–H_2O); mp 196 °C; ^{1}H-NMR ($CDCl_3$) δ 8.07 (d, 2H, J=8.4 Hz), 7.87 (d, 2H, J=8.4 Hz), 6.89 (m, 4H), 6.72 (dd, 1H, J=8.7, 2.9 Hz), 3.95 (s, 3H), 3.80 (s, 3H), 3.22 (s, 3H), 1.67 (m, 4H), 1.32 (s, 3H), 1.26 (s, 3H), 1.11 (s, 3H), 1.04 (s, 3H); *Anal.* Calcd for $C_{31}H_{34}N_2O_3$: C, 77.15; H, 7.10; N, 5.80. Found: C, 77.30; H, 6.96; N, 5.53.

A suspension of **15c** (50 mg, 0.10 mmol) in ethanol (4 ml) and 2 N NaOH (2 ml) was stirred at room temperature for 30 min. The mixture was poured into 2 N hydrochloric acid, and extracted with CH_2Cl_2. The organic layer was washed with water and brine, and dried over $MgSO_4$. After evaporation, the crude product (38 mg, 78.5%) was purified by recrystallization to give HX541 (**7e**). HX541 (**7e**): Orange prisms (EtOH–H_2O); mp 282 °C; ^{1}H-NMR ($CDCl_3$) δ 8.14 (d, 2H, J=8.8 Hz), 7.91 (d, 2H, J=8.4 Hz), 6.90 (m, 4H), 6.73 (dd, 1H, J=8.6, 2.9 Hz), 3.81 (s, 3H), 3.23 (s, 3H), 1.66 (m, 4H), 1.32 (s, 3H), 1.27 (s, 3H), 1.13 (s, 3H), 1.05 (s, 3H); *Anal.* Calcd for $C_{30}H_{32}N_2O_3$: C, 76.90; H, 6.88; N, 5.98. Found: C, 76.76; H, 7.02; N, 5.72.

HX543 (**7f**) was synthesized from **11c** and *n*-propyl iodide according to the above method. HX543 (**7f**): Orange powder (EtOH–H_2O); mp 257 °C; ^{1}H-NMR ($CDCl_3$) δ 8.15 (d, 2H, J=8.4 Hz), 7.91 (d, 2H, J=8.4 Hz), 6.90 (m, 4H), 6.74 (dd, 1H, J=8.8, 2.9 Hz), 3.80 (s, 3H), 3.57 (m, 2H), 1.65 (m, 6H), 1.31 (s, 3H), 1.26 (s, 3H), 1.13 (s, 3H), 1.04 (s, 3H), 0.91 (t, 3H, 7.3 Hz); *Anal.* Calcd for $C_{32}H_{36}N_2O_3$: C, 77.39; H, 7.31; N, 5.64. Found: C, 77.61; H, 7.20; N, 5.42.

4-(5*H*-2,3-(2,5-Dimethyl-2,5-hexano)-5-methyl-8-phenyldibenzo-[*b*,e][1,4]diazepin-11-yl)benzoic Acid (HX560, 7g) A solution of phenylboronic acid (26 mg, 0.21 mmol) in ethanol (1.5 ml) was added to a mixture of **18** (100 mg, 0.19 mmol) with tetrakis(triphenylphosphine)palladium (0) (6.6 mg) in benzene (8 ml) and 2 M sodium carbonate (0.19 ml) under an Ar atmosphere, and the whole was refluxed with vigorous stirring for 23 h. After cooling, 30% hydrogen peroxide (0.01 ml) was added. The mixture was stirred at room temperature for 1 h, and extracted with ether. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography to give **19** (36 mg, 36%), which was hydrolyzed under basic conditions to afford HX560 (**7g**, 97%). HX560 (**7g**): Red needles (EtOH–H_2O); mp>300 °C; ^{1}H-NMR ($CDCl_3$) δ 8.15 (d, 2H, J=8.8 Hz), 7.93 (d, 2H, J=8.4 Hz), 7.60 (m, 3H), 7.41 (m, 3H), 7.31 (t, 1H, J=7.3 Hz), 7.03 (d, 1H, J=8.1 Hz), 6.95 (s, 1H), 6.90 (s, 1H), 3.30 (s, 3H), 1.67 (m, 4H), 1.33 (s, 3H), 1.28 (s, 3H), 1.14 (s, 3H), 1.06 (s, 3H); *Anal.* Calcd for $C_{35}H_{34}N_2O_2$: C, 81.68; H, 6.66; N, 5.44. Found: C, 81.49; H, 6.74; N, 5.65.

4-(5*H*-10,11-Dihydro-2,3-(2,5-dimethyl-2,5-hexano)-5-methyldibenzo[*b*,*e*][1,4]-diazepin-11-yl)benzoic Acid (HX710, 8a) $NaBH_3CN$ (95%, 289 mg, 4.6 mmol) and 0.1 ml of TFA were added to a solution of **15d** (207 mg, 0.46 mmol) in 5 ml of methanol and 15 ml of methylene chroride. The mixture was stirred for 30 min, then poured into water, and extracted with methylene chloride. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to give crude **20**. **20**: ^{1}H-NMR ($CDCl_3$) δ 7.97 (d, 2H, J=8.4 Hz), 7.40 (d, 2H, J=8.1 Hz), 6.93 (s, 1H), 6.89 (s, 1H), 6.86 (dd, 1H, J=7.7, 1.5 Hz), 6.75 (m, 1H), 6.70 (m, 1H), 6.59 (dd, 1H, J=7.3, 1.8 Hz), 5.63 (s, 1H), 4.38 (br s, 1H), 3.90 (s, 3H), 2.96 (s, 3H), 1.64 (s, 4H), 1.25 (s, 3H), 1.23 (s, 3H), 1.20(s, 3H), 1.15 (s, 3H).

Compound **20** was hydrolyzed under basic conditions to afford HX710 (**8a**, 63%). HX710 (**8a**): Yellow powder (CH_2Cl_2–*n*-hexane); mp 239 °C; ^{1}H-NMR ($CDCl_3$) δ 7.99 (d, 2H, J=7.7 Hz), 7.37 (d, 2H, J=7.7 Hz), 6.92 (s, 1H), 6.90 (br s, 1H), 6.84 (dd, 1H, J=7.7, 1.5 Hz), 6.74 (td, 1H, J=8.1, 1.5 Hz), 6.68 (td, 1H, J=8.1, 1.5 Hz), 6.57 (d, 1H, J=8.0 Hz), 5.57 (s, 1H), 2.93 (s, 3H), 1.63 (s, 4H), 1.24 (s, 3H), 1.21 (s, 3H), 1.20(s, 3H), 1.15 (s, 3H); *Anal.* Calcd for $C_{29}H_{32}N_2O_2$: C, 79.06; H, 7.32; N, 6.36. Found: C, 78.85; H, 7.33; N, 6.40.

4-(5*H*-10,11-Dihydro-2,3-5,10-dimethyl-(2,5-dimethyl-2,5-hexano)-dibenzo[*b*,*e*][1,4]diazepin-11-yl)benzoic Acid (HX711, 8b) A solution of **20** (44 mg, 0.096 mmol) in DMF (4 ml) was added to a suspension of NaH (60%, 12 mg, 0.29 mmol) in DMF (1 ml), and the mixture was stirred for 10 min. Then, methyl iodide (0.03 ml) was added, and stirring was continued for 1 h. The mixture was poured into water, and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (AcOEt : *n*-hexane 1 : 40, then 1 : 20) to afford **21** (33 mg, 75%). **21**: ^{1}H-NMR ($CDCl_3$) δ 7.86 (d, 2H, J=8.4 Hz), 7.22 (d, 2H, J=8.1 Hz), 6.97 (s, 1 H), 6.92 (dd, 1H, J=8.1, 1.5 Hz), 6.90 (s, 1H), 6.87 (td, 1H, J=8.1, 1.5 Hz), 6.80 (td, 1H, J=8.1, 1.5 Hz), 6.72 (dd, 1H, J=8.1, 1.5 Hz), 3.87 (s, 3H), 3.04 (s, 3H), 2.98 (s, 3H), 1.63 (m, 4H), 1.26 (s, 3H), 1.23 (s, 3H), 1.21 (s, 3H), 1.14 (s, 3H).

Compound **21** was hydrolyzed under basic conditions to afford HX711 (**8b**, 63%). HX711 (**8b**): Yellow powder (CH_2Cl_2–*n*-hexane); mp 230 °C; ^{1}H-NMR ($CDCl_3$) δ 7.91 (d, 2H, J=8.4 Hz), 7.25 (d, 2H, J=8.4 Hz), 6.99 (s, 1H), 6.91 (md, 1H), 6.90 (s, 1H), 6.86 (dd, 1H, J=7.3, 1.5 Hz), 6.79 (td, 1H, J=8.1, 1.5 Hz), 6.73 (dd, 1H, J=7.3, 1.5 Hz), 3.01 (s, 3H), 2.99 (s, 3H), 1.63 (m, 4H), 1.26 (s, 3H), 1.23 (s, 3H), 1.22 (s, 3H), 1.16 (s, 3H); *Anal.* Calcd for $C_{30}H_{34}N_2O_2$: C, 79.26; H, 7.54; N, 6.16. Found: C, 79.10; H, 7.48; N, 5.92.

Compounds **8c**—**8e** were also synthesized according to the above method.

HX741 (8c): Orange powder (EtOH–H_2O); mp 248 °C; ^{1}H-NMR ($CDCl_3$) δ 7.92 (d, 2H, J=8.4 Hz), 7.27 (d, 2H, J=8.4 Hz), 7.01 (s, 1H), 6.87 (s, 1H), 6.85 (d, 1H, J=8.8 Hz), 6.32 (m, 2H), 5.21 (s, 1H), 3.73 (s, 3H), 3.02 (s, 3H), 2.96 (s, 3H), 1.62 (s, 4H), 1.25 (s, 3H), 1.24 (s, 3H), 1.22 (s, 3H), 1.17 (s, 3H); *Anal.* Calcd for $C_{31}H_{36}N_2O_3$: C, 76.83; H, 7.49; N, 5.78. Found: C, 75.98; H, 7.71; N,0 5.70.

HX743 (8d): Yellow needles (EtOH–H_2O); mp 262 °C; ^{1}H-NMR ($CDCl_3$) δ 7.95 (d, 2H, J=8.4 Hz), 7.41 (d, 2H, J=8.4 Hz), 7.13 (s, 1H), 6.83 (d, 1H, J=8.8 Hz), 6.83 (s, 1H), 6.35 (d, 1H, J=2.6 Hz), 6.25 (dd, 1H, J=8.4, 2.6 Hz), 5.17 (s, 1H), 3.75 (s, 3H), 3.33 (m, 2H), 3.05 (s, 3H), 1.58 (m, 4H), 1.24 (s, 3H), 1.22 (s, 3H), 1.20 (s, 3H), 1.19 (s, 3H), 0.96 (m, 2H), 0.69 (t, 3H, J=7.3 Hz); *Anal.* Calcd for $C_{33}H_{40}N_2O_3$: C, 77.31; H, 7.86; N, 5.46. Found: C, 77.46; H, 8.03; N, 5.21.

HX745 (8e): Pale yellow powder (EtOH–H_2O); mp 214 °C; ^{1}H-NMR ($CDCl_3$) δ 7.90 (d, 2H, J=8.4 Hz), 7.21 (d, 2H, J=8.4 Hz), 7.10 (s, 1H), 6.84 (s, 1H), 6.73 (d, 1H, J=8.1 Hz), 6.27 (m, 2H), 3.72 (s, 3H), 3.37 (m, 1H), 3.19 (m, 1H), 2.74 (s, 3H), 1.73 (m, 6H), 1.26 (s, 3H), 1.25 (s, 3H), 1.23 (s, 3H), 1.22 (s, 3H), 0.97 (t, 3H, J=7.3 Hz); *Anal.* Calcd for $C_{33}H_{40}N_2O_3$: C, 77.31; H, 7.86; N, 5.46. Found: C, 77.36; H, 8.15; N, 5.40.

Differentiation-Inducing Assay The human promyelocytic leukemia cell line HL-60[13,14] was provided by Prof. F. Takaku (Faculty of Medicine, University of Tokyo) in 1980, and has been maintained in continuous suspension culture. 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.

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 d and stained with Wright-Giemsa in order to check for morphological change. The percentages of differentiated cells were determined by NBT reduction assay as described.[15] 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.

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. The values in figures are the average of the two experiments. IC_{50} values of active compounds were calculated from the NBT reduction assay data by means of the van der Waerden method.[16]

Transactivation Assay Transient transactivation assays were carried out using COS-1 cells transfected with hRAR (α, β or γ) and DR5-tk-CAT or mRXR (α, β or γ) and DR1-tk-CAT, using retinoic acid (**1a**) or LGD1069 (**5**) as the activating ligand. The COS-1 cells were obtained from the Japanese Cancer Research Resources Bank (JCRB) and were maintained in Dulbecco's modified minimal essential medium (DMEM), supplemented with 5% FBS. For reporter gene assay, COS-1 cells were seeded in 24-well tissue culture plates at 5×10^4 cells per well with assay medium (5% FBS/DMEM). The cells were cultured at 37 °C in 5% CO_2 overnight and allowed to attach to the plates. Then, the medium were removed and transfection was performed by the standard calcium phosphate method. For each well, cells were transfected with 40—80 ng of receptor-expression plasmid, 200 ng of DR5G-TKCAT or DR1T-TKCAT, 80 ng of the reference plasmid pCMVβ (Clontech), and carrier plasmid pBluescript, to adjust the total DNA amount to 800 ng. After 6 h, cells were washed and fresh assay medium was added.

Each ligand was added as an EtOH solution (final 0.5% EtOH). After an additional 40—44 h of incubation, the cells were harvested, and CAT assay was perfomed with the CAT ELISA System (Boehringer Mannheim). The CAT activities were normalized to β-galactosidase activities. Assay was done in duplicate under each condition.

Acknowledgment This work was partially supported by a grant for the Promotion of Fundamental Studies in Health Science from the Organization for Pharmaceutical Safety and Research (OPSR).

References and Notes

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