Diarylamines Incorporating Hexahydrophenalene or Octahydrobenzoheptalene as Retinoid X Receptor (RXR)-Specific Agonists

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Selective ligands for retinoic acid receptors (RARs) and for retinoid X receptors (RXRs) are required for both biological studies and therapeutic purposes. We have synthesized a series of diarylamines incorporating hexahydrophenalene or octahydrobenzoheptalene as a hydrophobic moiety and examined their activities towards RARs and RXRs. Most of these compounds showed agonistic activity towards RXRs, but were inactive towards RARs. These RXR-specific ligands showed synergistic activity in RARα,β ligand-induced terminal differentiation of leukemia cell line HL-60.

Key words retinoid; retinoic acid receptor; retinoid X receptor (RXR); RXR-specific ligand

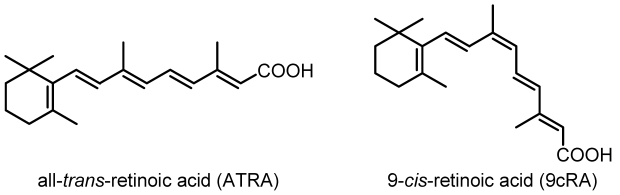
Retinoids are natural and synthetic analogues of all-*trans*-retinoic acid (ATRA), an active metabolite of vitamin A, and they modulate and regulate various biological functions, including cell differentiation, proliferation, and morphogenesis, by modulating gene transcription mediated by intranuclear retinoic acid receptors (RAR α, β, and γ) and retinoid X receptors (RXRα, β, and γ).[1] The endogenous ligands of RARs and RXRs have been identified as ATRA and 9-*cis*-retinoic acid (9cRA), respectively[2,3] (Fig. 1), though their specificity is not high. It is believed that the activation of RXRs alone does not cause any specific transactivation; rather RXRs act as heterodimers with RARs, peroxisome proliferator-activated receptors (PPARs), liver X receptor (LXR) and other nuclear receptors.[4,5] RXR-specific agonists alone cannot activate RXR-RAR heterodimers, but act as retinoid synergists that dose-dependently and robustly enhance the potency of RAR agonists. Thus, selective ligands specific for RARs and specific for RXRs are required for both biological studies and therapeutic purposes. Recently we reported several RARα and RARβ-specific ligands, *i.e.*, hexahydrophenalenyl- and octahydrobenzo[*ef*]heptalenyl-carbamoyl- or -carboxamido-4-benzoic acids, that do not activate RARγ or RXRs.[6] Here we report a series of diarylamines incorporating hexahydrophenalene and octahydrobenzoheptalene that show RXR-specific agonist activity.

An RXR ligand, LGD1069 (bexarotene), has been suggested to have a therapeutic effect in a mouse model of Alzheimer's disease (AD),[7] and our unpublished results also indicate that RXR ligands enhance the therapeutic effect of RAR ligands. Indeed, RXR ligands together with RAR ligands have potential therapeutic value in a variety of immunological and neurodegenerative disorders.[8–10]

Many synthetic RXR-selective agonists have been reported,[4,5] and diarylamine-type ligands, such as PA024, show very potent activity.[11] Therefore, building on the structures of our previously developed potent and subtype-selective RAR agonists, in the present work we synthesized a series of diarylamine ligands incorporating a tricyclic hydrophobic moiety, *i.e.*, hexahydrophenalene or octahydrobenzoheptalene, as candidate RXR agonists. The target compounds were synthesized as follows. After coupling reaction of tricyclic amine and ethyl 4-iodobenzoate catalyzed by $Pd(dba)_3$ in the presence of (±)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP), *N*-alkylation followed by ester hydrolysis afforded diphenylamine compounds **1a**–**d**, **2a**–**d**, and **3a**–**f** (Fig. 2). On the other hand, after treatment of tricyclic amine with ethyl 2-chloropyrimidine-5-carboxylate in the presence of potassium carbonate, *N*-alkylation followed by ester hydrolysis afforded (*N*-arylamino)pyrimidine compounds **1g**–**j**, **2g**–**j**, and **3g**–**j**.

The activities of compounds **1a**–**3j** were evaluated by means of reporter assay utilizing COS-1 cells transfected with expression vectors containing GAL4-RARα, β, or γ and full-length RXRα, β, or γ. *N*-Unsubstituted compounds (*e.g.* **1a**, **2a**, **3a**) and some *N*-methyl compounds (*e.g.* **1b**, **2b**, **3b**) showed moderate agonistic activity towards RARα and β, but most of the *N*-alkylated compounds had no activity towards RARs. On the other hand, most of the compounds showed agonistic activity towards RXRs, and in particular, *N*-ethyl- and *N*-cyclopropylmethyl-pyrimidine-5-carboxylic acid compounds (*e.g.* **1j**, **2i**, **2j**, **3i**, **3j**) showed potent and selective activity towards RXRα (Table 1).

Several of the compounds prepared were potent transactivators in the cell-based assay. Substitution on the nitrogen atom with a medium-sized alkyl group (more than two carbon atoms) is necessary for significant RXR activity. When the substituent is a hydrogen or methyl group (**1a**, **b**, **2a**, **b**, **3a**, **b**), RXR activity is weak, but at the same time significant RAR activity was observed. These latter compounds are similar to LGD1069, which is generally accepted to be an RXR ligand with weak RAR activity. The more potent compounds bearing a pyrimidinylcarboxylic acid moiety showed similar activity to PA024, which also bears this structure. Introduction of tricy-



all-*trans*-retinoic acid (ATRA) 9-*cis*-retinoic acid (9cRA)

Fig. 1. The Structures of Endogenous RAR and RXR Ligands

The authors declare no conflict of interest.

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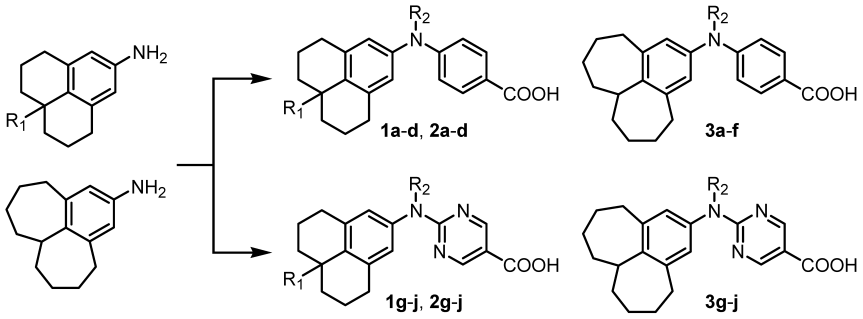


	R_1	R_2		R_1	R_2		R_2
1a	H	H	**2a**	Me	H	**3a**	H
1b	H	Me	**2b**	Me	Me	**3b**	Me
1c	H	Et	**2c**	Me	Et	**3c**	Et
1d	H	$CH_2{}^cPr$	**2d**	Me	$CH_2{}^cPr$	**3d**	$CH_2{}^cPr$
						3e	^iBu
						3f	Bn
1g	H	H	**2g**	Me	H	**3g**	H
1h	H	Me	**2h**	Me	Me	**3h**	Me
1i	H	Et	**2i**	Me	Et	**3i**	Et
1j	H	$CH_2{}^cPr$	**2j**	Me	$CH_2{}^cPr$	**3j**	$CH_2{}^cPr$

Fig. 2. The Structures of Diarylamine Derivatives

Table 1. Bioactivities of Diarylamine Derivatives

Compound[a]	Transcriptional activation assay data[b]									HL-60 Differentiation[c]	
	RARα 1000 nM	RARβ 1000 nM	RARγ 1000 nM	RXRα		RXRβ		RXRγ		Alone	With Am80 1 nM
				100 nM	1000 nM	100 nM	1000 nM	100 nM	1000 nM		
Blank	1	1	1	1	1	1	1	1	1	1	16
9cRA	71[d]	63[d]	73[d]	10[d]	20[d]	4[d]	13[d]	5[d]	10[d]	N.D.[e]	N.D.[e]
HX630	N.D.[e]	N.D.[e]	N.D.[e]	2	35	1	26	6	31	2	73
PA024	N.D.[e]	N.D.[e]	N.D.[e]	53	54	38	38	35	35	5[d]	86
1a	31	17	2	1[d]	11	1[d]	3	2[d]	12[d]	5	N.D.[e]
1b	3	4	1	1[d]	42[d]	1[d]	8[d]	1[d]	21[d]	N.D.[e]	N.D.[e]
1c	1	2	1	2	60	1	43	2	41	1	26
1d	1	2	1	25	57	7	41	15	37	3	77
1g	2	5	1	1[d]	2[d]	1[d]	1[d]	1[d]	2[d]	N.D.[e]	N.D.[e]
1h	1	1	1	1[d]	42	1[d]	1[d]	1[d]	17[d]	N.D.[e]	N.D.[e]
1i	1	1	1	9	53	2[d]	29[d]	6[d]	30[d]	N.D.[e]	N.D.[e]
1j	1	1	1	39	58	5[d]	31[d]	16[d]	36[d]	3	53
2a	31	22	4	1	4	1[d]	2	2[d]	6[d]	10	N.D.[e]
2b	9	11	1	1[d]	42[d]	1[d]	7[d]	2[d]	19[d]	N.D.[e]	N.D.[e]
2c	2	4	1	33[d]	64	3[d]	33	22[d]	39[d]	N.D.[e]	N.D.[e]
2d	2	4	1	32	57	4	37	26	38	N.D.[e]	N.D.[e]
2g	8	12	2	1[d]	2[d]	1[d]	1[d]	1[d]	1[d]	N.D.[e]	N.D.[e]
2h	1	2	1	3[d]	52[d]	1[d]	9[d]	3[d]	25[d]	N.D.[e]	N.D.[e]
2i	1	2	1	35	52	5[d]	31[d]	17[d]	30[d]	3	56
2j	1	2	1	47	52	13[d]	30[d]	25[d]	30[d]	5	76
3a	22	15	4	N.D.[e]	7[d]	1[d]	4	2[d]	10[d]	N.D.[e]	N.D.[e]
3b	5	9	2	N.D.[e]	13[d]	1[d]	7	3[d]	22[d]	N.D.[e]	N.D.[e]
3c	1	3	1	32	50	7	38	19	33	2	85
3d	2	3	1	26	51	6	34	18	33	2	88
3e	2	3	1	21	44	5[d]	27[d]	15[d]	26[d]	N.D.[e]	N.D.[e]
3f	2	3	1	1	11	1[d]	2[d]	1[d]	9[d]	N.D.[e]	N.D.[e]
3g	10	10	2	3[d]	39[d]	1[d]	8[d]	3[d]	17[d]	N.D.[e]	N.D.[e]
3h	2	3	1	22[d]	54[d]	3[d]	22[d]	13[d]	29[d]	N.D.[e]	N.D.[e]
3i	1	2	1	47	51	14[d]	32[d]	22[d]	27[d]	2	91
3j	1	2	1	42	51	6[d]	27[d]	18[d]	29[d]	4	87

a) Structures of **1a**–**3j** are shown in Fig. 2. *b*) Data are mean values of fold induction (*vs.* DMSO) at 100 or 1000 nM measured by two or more experiments. *c*) Data are mean values of the percentage of differentiated cells evaluated from NBT reduction assay at 100 nM. *d*) One experiment. *e*) Not determined.

clic hydrocarbon (hexahydrophenalene or octahydrobenzoheptalene) instead of bicyclic hydrocarbon (tetramethyltetraline) as a hydrophobic moiety seems to result in slight selectivity for RXRα (**1j**, **2j**, **3j** *vs.* PA024). Although it is not clear how the RXR subtype-selectivity arises, it appears that structural extension of tricyclic hydrocarbon may have the potential to increase selectivity for RXR subtypes. This is of interest, because few RXR subtype-selective ligands have been developed.[4,5] These compounds do not show any activity towards RARs even at concentrations above 1000 nM, and are thus activators of RXRs. Dose–response curves of representative compounds are shown in Fig. 3.

These RXR ligands have a synergistic effect on RAR ligand-induced terminal differentiation of leukemia cell line HL-60.[11,12] The *N*-unsubstituted compounds showed weak cell-differentiating activity on their own, *i.e.*, they act as weak RAR agonists. However, the *N*-substituted compounds themselves showed no RAR agonist activity, but in the presence of a low concentration (1 nM) of RARα, β ligand Am80, these new RXR ligands showed synergistic differentiation-inducing activity in this system. This is consistent with our recent finding that the RXR ligand HX630 potently enhances RAR ligand activities in Alzheimer's model animals (manuscript submitted).

In conclusion, building on the structures of our previously reported potent synthetic retinoids, we synthesized a series of diarylamines incorporating hexahydrophenalene or octahydrobenzoheptalene as a bulky hydrophobic moiety. Most of these compounds showed agonistic activity towards RXRs, but were inactive towards RARs. Thus, these RXR ligands act as retinoid synergists. Some of them, such as **2i**, **j**, and **3i**, **j** (Fig. 4), are considered to be potential biological tools and candidate therapeutic drugs, even though they are somewhat less potent than the strongest known RXR ligand, PA024. They are more potent than the most widely used RXR ligand, HX630, and more RXR-selective than LGD1069 and LGD100268.

Experimental

General Melting points were determined on a Yanagimoto micro-melting point apparatus (hot plate), and are not corrected. ^{1}H-NMR spectra were obtained on a Varian Mercury 300 at 300 MHz in $CDCl_3$ or in DMSO-d_6. The chemical shifts are calculated on the basis of tetramethylsilane (0 ppm for ^{1}H-NMR in $CDCl_3$) or $(\underline{CH}_3)_2SO$ (2.49 ppm in DMSO-d_6). High resolution-electrospray ionization-mass spectra (HR-ESI-MS) was recorded on a Shimadzu LCMS-IT-TOF spectrom-

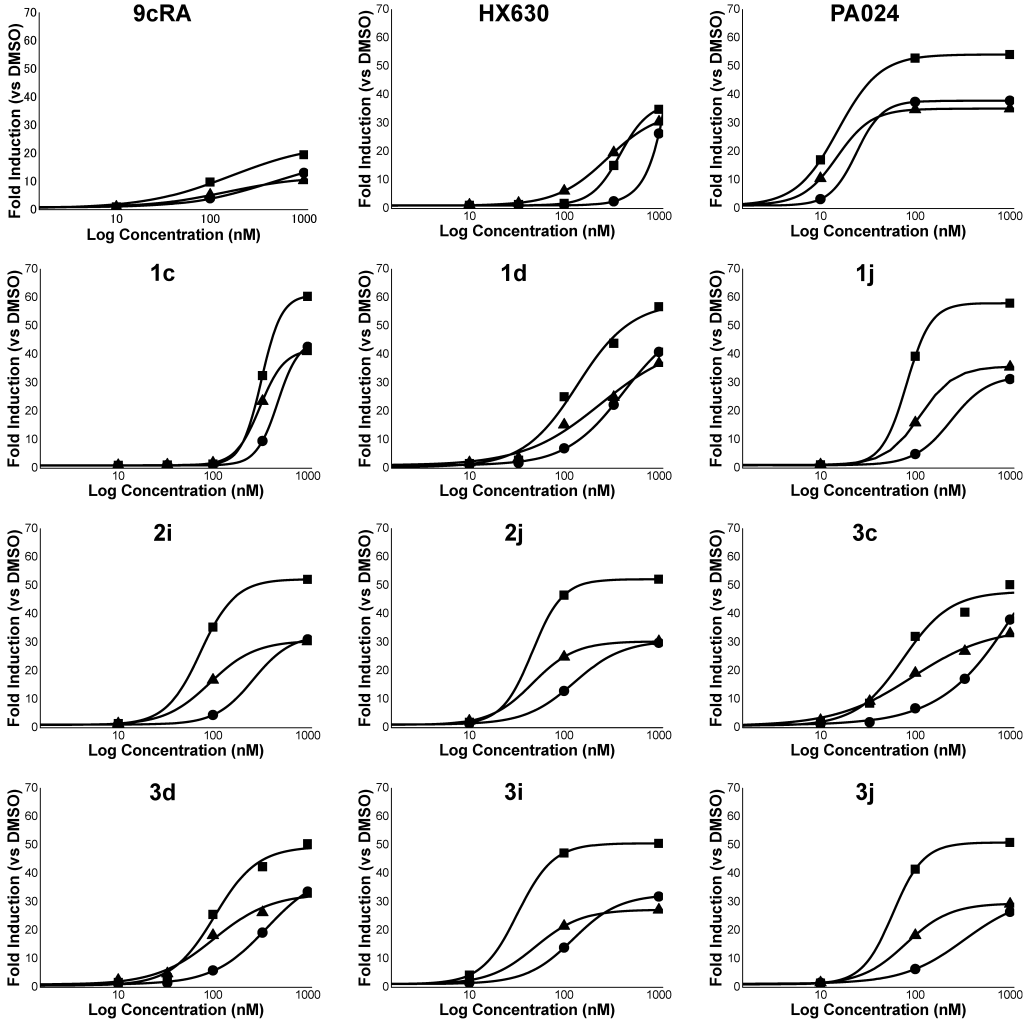
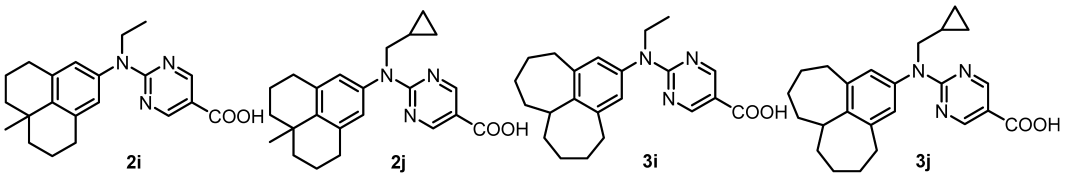


Fig. 3. Transcriptional Activities Measured by Means of Reporter Gene Assay in Cos-1 Cells with Full Length RXRα (■), RXRβ (●), and RXRγ (▲)

Data are mean values of fold increase (*vs.* DMSO). The horizontal scale is the molar concentration of added compound.



2i 2j 3i 3j

Fig. 4. The Structures of Synthetic RXR Ligands (**2i**, **2j**, **3i**, and **3j**)

eter. Column chromatography was conducted on silica gel (Fuji Davison BW 200). Usual work-up refers to washing of the organic phase with water or brine, drying over anhydrous Na_2SO_4, and removal of the solvent(s) by evaporation under reduced pressure.

4-[*N*-(5,6,6a,7,8,9-Hexahydro-4*H*-2-phenalenyl)amino]-benzoic Acid (1a) To a solution of 5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenylamine[6] (0.250 g, 1.33 mmol) and ethyl 4-iodobenzoate (0.360 g, 1.30 mmol) in benzene (5 mL) was added $Pd_2(dba)_3$ (0.024 g, 0.0262 mmol), (±)-BINAP (0.036 g, 0.0578 mmol), and Cs_2CO_3 (0.455 g, 1.40 mmol), and the mixture was stirred overnight at 80°C under an Ar atmosphere. After cooling, the mixture was acidified by adding 2 M aqueous HCl and extracted with Et_2O. Usual work-up gave a residue, which was purified by silica gel column chromatography (AcOEt–*n*-hexane=1 : 50) to afford ethyl 4-[*N*-(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]benzoate (0.160 g, 36%). To a suspension of the above ester (0.154 g, 0.459 mmol) in EtOH (12 mL) was added 20% aqueous NaOH (3 mL) and the mixture was stirred overnight at rt. The reaction mixture was acidified by adding 2 M aqueous HCl and extracted with $CHCl_3$. Usual work-up gave a residue, which was purified by recrystallization from AcOEt–*n*-hexane to give **1a** (0.125 g, 89%). **1a**: Pale brown needles (AcOEt–*n*-hexane); mp 204–205°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26–1.40 (2H, m), 1.72–1.86 (2H, m), 1.88–2.00 (4H, m), 2.50–2.60 (1H, m), 2.77–2.81 (4H, m), 5.93 (1H, br s), 6.76 (2H, s), 6.94 (2H, d, J=8.7 Hz), 7.95 (2H, d, J=8.7 Hz) ; HR-MS Calcd for $C_{20}H_{21}NO_2$ ([M+H]$^+$): 308.1651. Found: 308.1651.

Other diphenylamine compounds (**2a**, **3a**) were synthesized similarly.

4-[*N*-(6a-Methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)-amino]benzoic Acid (**2a**): Pale brown needles (AcOEt–*n*-hexane); mp 232–234°C; ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, s), 1.51 (2H, td, J=12.9, 4.8 Hz), 1.69 (2H, dt, J=12.9, 4.1 Hz), 1.76–1.87 (2H, m), 1.99–2.18 (2H, m), 2.76 (2H, dt, J=17.3, 8.7 Hz), 2.89 (2H, ddd, J=17.3, 7.9, 3.3 Hz), 5.91 (1H, br s), 6.74 (2H, s), 6.94 (2H, d, J=8.6 Hz), 7.94 (2H, d, J=8.6 Hz); HR-MS Calcd for $C_{21}H_{23}NO_2$ ([M+H]$^+$): 322.1807. Found: 322.1808.

4-[*N*-(5,6,7,7a,8,9,10,11-Octahydro-4*H*-2-benzo[*ef*]-heptalenyl)amino]benzoic Acid (**3a**): Brown needles (AcOEt–*n*-hexane); mp 203–204°C; ^{1}H-NMR ($CDCl_3$) δ: 1.48–1.67 (6H, m), 1.80–1.96 (6H, m), 2.74–2.87 (4H, m), 3.18–3.25 (1H, m), 5.92 (1H, br s), 6.78 (2H, s), 6.95 (2H, d, J=8.7 Hz), 7.95 (2H, d, J=8.7 Hz); HR-MS Calcd for $C_{22}H_{26}NO_2$ ([M+H]$^+$): 336.1964. Found: 336.1965.

4-[*N*-Methyl-(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)-amino]benzoic Acid (1b) To a suspension of NaH (60%, 0.012 g, 0.300 mmol) in *N*,*N*-dimethylformamide (DMF) (3 mL) was added a solution of ethyl 4-[*N*-(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]benzoate (0.030 g, 0.0976 mmol) in DMF (2 mL) and the whole mixture was stirred at rt for 30 min. MeI (0.020 mL, 0.321 mmol) was added to the whole mixture and the reaction mixture was stirred overnight at ambient temperature. The reaction mixture was poured into saturated aqueous NH_4Cl and extracted with Et_2O. Usual work-up gave a residue, which was purified by silica gel column chromatography (AcOEt–*n*-hexane=1 : 80) to afford ethyl 4-[*N*-methyl-(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)-amino]benzoate (0.033 g, quant.). To a suspension of the above ester (0.033 g, 0.0976 mmol) in EtOH (10 mL) was added 20% aqueous NaOH (1 mL) and the mixture was stirred overnight at 50°C. The mixture was acidified by adding 2 M aqueous HCl and extracted with $CHCl_3$. Usual work-up gave a residue, which was purified by recrystallization from EtOH–$CHCl_3$ to afford **1b** (0.027 g, 87%). **1b**: Colorless needles (EtOH–$CHCl_3$); mp 288–289°C; ^{1}H-NMR ($CDCl_3$) δ: 1.29–1.42 (2H, m), 1.72–1.86 (2H, m), 1.88–2.01 (4H, m), 2.54–2.62 (1H, m), 2.75–2.80 (4H, m), 3.31 (3H, s), 6.72 (2H, d, J=9.0 Hz), 6.75 (2H, s), 7.85 (2H, d, J=9.0 Hz); HR-MS Calcd for $C_{21}H_{24}NO_2$ ([M+H]$^+$): 322.1807. Found: 322.1805.

Other *N*-alkyl-diphenylamine compounds (**1c**, **d**, **2b**–**d**, **3b**–**f**) were synthesized similarly.

4-[*N*-Ethyl-(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]-benzoic Acid (**1c**): Colorless needles (EtOH); mp 284–285°C; ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.2 Hz), 1.29–1.43 (2H, m), 1.76–1.86 (2H, m), 1.88–2.00 (4H, m), 2.53–2.64 (1H, m), 2.77–2.81 (4H, m), 3.75 (2H, q, J=7.2 Hz), 6.65 (2H, d, J=8.7 Hz), 6.73 (2H, s), 7.85 (2H, d, J=8.7 Hz); HR-MS Calcd for $C_{22}H_{26}NO_2$ ([M+H]$^+$): 336.1964. Found: 336.1941.

4-[*N*-Cyclopropylmethyl-(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]benzoic Acid (**1d**): Colorless needles ($CHCl_3$–*n*-hexane); mp 214–216°C; ^{1}H-NMR ($CDCl_3$) δ: 0.17 (2H, q, J=5.7 Hz), 0.48–0.54 (2H, m), 1.14–1.22 (1H, m), 1.29–1.43 (2H, m), 1.75–1.86 (2H, m), 1.89–2.00 (4H, m), 2.53–2.62 (1H, m), 2.77–2.80 (4H, m), 3.55 (2H, d, J=6.3 Hz), 6.68 (2H, d, J=8.7 Hz), 6.75 (2H, s), 7.86 (2H, d, J=8.7 Hz); HR-MS Calcd for $C_{24}H_{28}NO_2$ ([M+H]$^+$): 362.2120. Found: 362.2111.

4-[*N*-Methyl-(6a-methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]benzoic Acid (**2b**): Colorless needles (EtOH); mp 257–258°C; ^{1}H-NMR (DMSO-d_6) δ: 1.11 (3H, s), 1.40 (2H, td, J=12.9, 4.8 Hz), 1.59–1.66 (2H, m), 1.68–1.78 (2H, m), 1.89–2.04 (2H, m), 2.68 (2H, dt, J=17.4, 8.7 Hz), 2.82 (2H, ddd, J=17.4, 8.1, 3.3 Hz), 3.21 (3H, s), 6.68 (2H, d, J=8.7 Hz), 6.70 (2H, s), 7.69 (2H, d, J=8.7 Hz); HR-MS Calcd for $C_{22}H_{26}NO_2$ ([M+H]$^+$): 336.1964. Found: 336.1959.

4-[*N*-Ethyl-(6a-methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]benzoic Acid (**2c**): Colorless needles (EtOH); mp 279–280°C; ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, s), 1.24 (3H, t, J=6.9 Hz), 1.48–1.58 (2H, m), 1.66–1.73 (2H, m), 1.76–1.88 (2H, m), 1.99–2.16 (2H, m), 2.76 (2H, dt, J=17.1, 8.7 Hz), 2.88 (2H, ddd, J=17.1, 8.1, 3.3 Hz), 3.74 (2H, q, J=6.9 Hz), 6.64 (2H, d, J=8.7 Hz), 6.70 (2H, s), 7.86 (2H, d, J=8.7 Hz); HR-MS Calcd for $C_{23}H_{28}NO_2$ ([M+H]$^+$): 350.2120.

Found: 350.2111.

4-[*N*-Cyclopropylmethyl-(6a-methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]benzoic Acid (**2d**): Colorless needles ($CHCl_3$–*n*-hexane); mp 234–235°C; ^{1}H-NMR ($CDCl_3$) δ: 0.16 (2H, q, J=4.8 Hz), 0.47–0.53 (2H, m), 1.12–1.17 (1H, m), 1.19 (3H, s), 1.48–1.58 (2H, m), 1.67–1.72 (2H, m), 1.76–1.87 (2H, m), 1.99–2.16 (2H, m), 2.75 (2H, dt, J=17.4, 8.7 Hz), 2.83–2.92 (2H, m), 3.54 (2H, d, J=6.3 Hz), 6.67 (2H, d, J=8.7 Hz), 6.73 (2H, s), 7.86 (2H, d, J=8.7 Hz); HR-MS Calcd for $C_{25}H_{30}NO_2$ ($[M+H]^+$): 376.2277. Found: 376.2281.

4-[*N*-Methyl-(5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]-heptalenyl)amino]benzoic Acid (**3b**): Colorless needles (AcOEt–*n*-hexane); mp 244–245°C; ^{1}H-NMR ($CDCl_3$) δ: 1.53–1.71 (6H, m), 1.81–1.97 (6H, m), 2.73–2.89 (4H, m), 3.20–3.28 (1H, m), 3.34 (3H, s), 6.73 (2H, d, J=8.7 Hz), 6.78 (2H, s), 7.90 (2H, d, J=8.7 Hz); HR-MS Calcd for $C_{23}H_{28}NO_2$ ($[M+H]^+$): 350.2120. Found: 350.2139.

4-[*N*-Ethyl-(5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]-heptalenyl)amino]benzoic Acid (**3c**): Colorless needles (AcOEt–*n*-hexane); mp 256.5–257.5°C; ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.2 Hz), 1.52–1.72 (6H, m), 1.81–1.97 (6H, m), 2.73–2.89 (4H, m), 3.21–3.28 (1H, m), 3.76 (2H, q, J=7.2 Hz), 6.65 (2H, d, J=8.7 Hz), 6.75 (2H, s), 7.87 (2H, d, J=8.7 Hz); HR-MS Calcd for $C_{24}H_{30}NO_2$ ($[M+H]^+$): 364.2277. Found: 364.2289.

4-[*N*-Cyclopropylmethyl-(5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]heptalenyl)amino]benzoic Acid (**3d**): Colorless needles (AcOEt–*n*-hexane); mp 222–223°C; ^{1}H-NMR ($CDCl_3$) δ: 0.15 (2H, dd, J=10.8, 5.1 Hz), 0.47–0.53 (2H, m), 1.13–1.22 (1H, m), 1.52–1.71 (6H, m), 1.80–1.97 (6H, m), 2.73–2.88 (4H, m), 3.21–3.28 (1H, m), 3.56 (2H, d, J=6.3 Hz), 6.69 (2H, d, J=9.0 Hz), 6.78 (2H, s), 7.88 (2H, d, J=9.0 Hz); HR-MS Calcd for $C_{26}H_{32}NO_2$ ($[M+H]^+$): 390.2433. Found: 390.2435.

4-[*N*-Isobutyl-(5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]-heptalenyl)amino]benzoic Acid (**3e**): Colorless needles (EtOH); mp 250–251°C; ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, s), 0.98 (3H, s), 1.53–1.69 (6H, m), 1.80–1.98 (6H, m), 2.03–2.12 (1H, m), 2.72–2.87 (4H, m), 3.20–3.28 (1H, m), 3.51 (2H, d, J=7.5 Hz), 6.67 (2H, d, J=9.3 Hz), 6.74 (2H, s), 7.85 (2H, d, J=9.3 Hz); HR-MS Calcd for $C_{26}H_{34}NO_2$ ($[M+H]^+$): 392.2590. Found: 392.2586.

4-[*N*-Benzyl-(5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]-heptalenyl)amino]benzoic Acid (**3f**): Colorless needles (EtOH); mp 228–229°C; ^{1}H-NMR ($CDCl_3$) δ: 1.51–1.67 (6H, m), 1.79–1.93 (6H, m), 2.71–2.87 (4H, m), 3.19–3.26 (1H, m), 4.99 (2H, s), 6.71 (2H, d, J=9.3 Hz), 6.86 (2H, s), 7.23–7.33 (5H, m), 7.82 (2H, d, J=9.3 Hz); HR-MS Calcd for $C_{29}H_{32}NO_2$ ($[M+H]^+$): 426.2433. Found: 426.2432.

2-[*N*-(5,6,6a,7,8,9-Hexahydro-4*H*-2-phenalenyl)-amino]pyrimidine-5-carboxylic Acid (1g) A mixture of 5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenylamine (0.100 g, 0.531 mmol), ethyl 2-chloropyrimidine-5-carboxylate (0.101 g, 0.541 mmol), and K_2CO_3 (0.400 g) was heated overnight at 110°C. After cooling, the reaction mixture was poured into water, and extracted with $CHCl_3$. Usual work-up gave a residue, which was purified by silica gel column chromatography (AcOEt–*n*-hexane=1 : 20) to afford ethyl 2-[*N*-(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]pyrimidine-5-carboxylate (0.167 g, 93%). To a suspension of the above ester (0.055 g, 0.163 mmol) in EtOH (5 mL) was added 20% aqueous NaOH (1 mL) and the mixture was stirred at 50°C for 3 h. The reaction was acidified by adding 2 M aqueous HCl and extracted with $CHCl_3$. Usual work-up gave a residue, which was purified by recrystallization from EtOH–$CHCl_3$ to give **1g** (0.045 g, 90%). **1g**: Colorless needles (EtOH–$CHCl_3$); mp 296–298°C; ^{1}H-NMR (DMSO-d_6) δ: 1.14–1.23 (2H, m), 1.63–1.71 (2H, m), 1.77–1.90 (4H, m), 2.22–2.28 (1H, m), 2.65–2.70 (4H, m), 7.17 (2H, s), 8.66 (2H, s), 9.75 (1H, s); HR-MS Calcd for $C_{18}H_{20}N_3O_2$ ($[M+H]^+$): 310.1556. Found: 310.1538.

Other pyrimidinecarboxylic acid compounds (**2g**, **3g**) were synthesized similarly.

2-[*N*-(6a-Methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)-amino]pyrimidine-5-carboxylic Acid (**2g**): Colorless needles (EtOH–$CHCl_3$); mp >300°C; ^{1}H-NMR (DMSO-d_6) δ: 1.06 (3H, s), 1.30–1.40 (2H, m), 1.56–1.63 (2H, m), 1.66–1.75 (2H, m), 1.91–1.98 (2H, m), 2.62–2.71 (2H, m), 2.74–2.78 (2H, m), 7.13 (2H, s), 8.76 (2H, s), 9.78 (1H, s); HR-MS Calcd for $C_{19}H_{22}N_3O_2$ ($[M+H]^+$): 324.1712. Found: 324.1698.

2-[*N*-(5,6,7,7a,8,9,10,11-Octahydro-4*H*-2-benzo[*ef*]-heptalenyl)amino]pyrimidine-5-carboxylic Acid (**3g**): Colorless needles (EtOH–$CHCl_3$); mp >300°C; ^{1}H-NMR (DMSO-d_6) δ: 1.38–1.55 (6H, m), 1.71–1.88 (6H, m), 2.66–2.81 (4H, m), 3.11–3.20 (1H, m), 7.26 (2H, s), 8.79 (2H, s), 9.85 (1H, s); HR-MS Calcd for $C_{20}H_{24}N_3O_2$ ($[M+H]^+$): 338.1869. Found: 338.1870.

2-[*N*-Methyl-(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)-amino]pyrimidine-5-carboxylic Acid (1h) To a suspension of NaH (60%, 0.008 g, 0.200 mmol) in DMF (2 mL) was added a solution of ethyl 2-[*N*-(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]pyrimidine-5-carboxylate (0.032 g, 0.0948 mmol) in DMF (1 mL) and the mixture was stirred for 10 min at rt. MeI (0.015 mL, 0.241 mmol) was added, and the reaction mixture was stirred overnight at ambient temperature. The mixture was poured into saturated aqueous NH_4Cl and extracted with Et_2O. Usual work-up gave a residue, which was suspended in EtOH (5 mL). To this suspension was added 20% aqueous NaOH (1 mL) and the mixture was stirred at 50°C for 3 h, then acidified by adding 2 M aqueous HCl and extracted with $CHCl_3$. Usual work-up gave a residue, which was purified by recrystallization from EtOH to afford **1h** (0.027 g, 87%). **1h**: Colorless needles (EtOH); mp 255–256°C; ^{1}H-NMR ($CDCl_3$) δ: 1.34–1.43 (2H, m), 1.72–1.86 (2H, m), 1.88–1.95 (4H, m), 2.53–2.62 (1H, m), 2.80–2.84 (4H, m), 3.55 (3H, s), 6.81 (2H, s), 8.91 (2H, s); HR-MS Calcd for $C_{19}H_{22}N_3O_2$ ($[M+H]^+$): 324.1712. Found: 324.1688.

Other *N*-alkyl-pyrimidinecarboxylic acid compounds (**1i**, **j**, **2h**–**j**, **3h**–**j**) were synthesized similarly.

2-[*N*-Ethyl-(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)-amino]pyrimidine-5-carboxylic Acid (**1i**): Colorless needles ($CHCl_3$–*n*-hexane); mp 252–253°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.2 Hz), 1.34–1.43 (2H, m), 1.75–1.85 (2H, m), 1.89–1.98 (4H, m), 2.54–2.63 (1H, m), 2.80–2.84 (4H, m), 4.02 (2H, q, J=7.2 Hz), 6.76 (2H, s), 8.89 (2H, s); HR-MS Calcd for $C_{20}H_{24}N_3O_2$ ($[M+H]^+$): 338.1869. Found: 338.1856.

2-[*N*-Cyclopropylmethyl-(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]pyrimidine-5-carboxylic Acid (**1j**): Pale yellow amorphous solid; ^{1}H-NMR ($CDCl_3$) δ: 0.25 (2H, dd, J=10.8, 4.8 Hz), 0.44–0.50 (2H, m), 1.15–1.23 (1H, m), 1.30–1.43 (2H, m), 1.75–1.85 (2H, m), 1.89–1.98 (4H, m), 2.54–2.62 (1H, m), 2.82 (4H, brdd, J=8.1, 4.5 Hz), 3.86 (2H, d, J=7.2 Hz), 6.80 (2H, s), 8.79 (2H, s); HR-MS Calcd for $C_{22}H_{25}N_3O_2$ ($[M+H]^+$): 364.2025. Found: 364.2009.

2-[*N*-Methyl-(6a-methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]pyrimidine-5-carboxylic Acid (**2h**): Colorless needles (EtOH); mp >300°C; ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, s), 1.52 (2H, td, J=12.9, 5.4 Hz), 1.67 (2H, m), 1.77–1.86 (2H, m), 1.99–2.15 (2H, m), 2.74–2.86 (2H, m), 2.91 (2H, ddd, J=17.1, 7.8, 3.3 Hz), 3.54 (3H, s), 6.80 (2H, s), 8.91 (2H, s); HR-MS Calcd for $C_{20}H_{24}N_3O_2$ ($[M+H]^+$): 338.1869. Found: 338.1845.

2-[*N*-Ethyl-(6a-methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]pyrimidine-5-carboxylic Acid (**2i**): Colorless needles ($CHCl_3$–*n*-hexane); mp 257–258°C; ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, s), 1.24 (3H, t, J=7.2 Hz), 1.53 (2H, td, J=12.9, 4.8 Hz), 1.63–1.70 (2H, m), 1.77–1.86 (2H, m), 2.01–2.10 (2H, m), 2.74–2.83 (2H, m), 2.86–2.95 (2H, m), 4.01 (2H, q, J=7.2 Hz), 6.73 (2H, s), 8.89 (2H, s); HR-MS Calcd for $C_{21}H_{26}N_3O_2$ ($[M+H]^+$): 352.2025. Found: 352.2008.

2-[*N*-Cyclopropylmethyl-(6a-methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)amino]pyrimidine-5-carboxylic Acid (**2j**): Colorless needles (EtOH–*n*-hexane); mp 123–124°C; ^{1}H-NMR ($CDCl_3$) δ: 0.20–0.25 (2H, m), 0.43–0.49 (2H, m), 0.95–0.98 (1H, m), 1.18 (3H, s), 1.48–1.58 (2H, m), 1.63 (2H, m), 1.74–1.85 (2H, m), 1.98–2.10 (2H, m), 2.74–2.82 (2H, m), 2.83–2.95 (2H, m), 3.85 (2H, d, J=6.9 Hz), 6.77 (2H, s), 8.87 (2H, s); HRMS Calcd for $C_{23}H_{28}N_3O_2$ ($[M+H]^+$): 378.2182. Found: 378.2163.

2-[*N*-Methyl-(5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]-heptalenyl)amino]pyrimidine-5-carboxylic Acid (**3h**): Colorless needles (EtOH); mp >300°C; ^{1}H-NMR ($CDCl_3$) δ: 1.54–1.69 (6H, m), 1.82–1.98 (6H, m), 2.75–2.93 (4H, m), 3.20–3.28 (1H, m), 3.56 (3H, s), 6.85 (2H, s), 8.91 (2H, s); HR-MS Calcd for $C_{21}H_{26}N_3O_2$ ($[M+H]^+$): 352.2025. Found: 352.2006.

2-[*N*-Ethyl-(5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]-heptalenyl)amino]pyrimidine-5-carboxylic Acid (**3i**): Colorless needles (EtOH); mp 266–267°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.2 Hz), 1.54–1.69 (6H, m), 1.82–1.94 (6H, m), 2.75–2.92 (4H, m), 3.21–3.28 (1H, m), 4.12 (2H, q, J=7.2 Hz), 6.79 (2H, s), 8.89 (2H, s); HR-MS Calcd for $C_{22}H_{28}N_3O_2$ ($[M+H]^+$): 366.2182. Found: 366.2160.

2-[*N*-Cyclopropylmethyl-(5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]heptalenyl)amino]pyrimidine-5-carboxylic Acid (**3j**): Colorless needles (*n*-hexane); mp 174–176°C; ^{1}H-NMR ($CDCl_3$) δ: 0.22 (2H, dd, J=10.8, 4.5 Hz), 0.43–0.50 (2H, m), 1.14–1.20 (1H, m), 1.57–1.68 (6H, m), 1.85–1.97 (6H, m), 2.75–2.91 (4H, m), 3.21–3.29 (1H, m), 3.87 (2H, d, J=6.9 Hz), 6.83 (2H, s), 8.88 (2H, s); HR-MS Calcd for $C_{24}H_{30}N_3O_2$ ($[M+H]^+$): 392.2338. Found: 392.2325.

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References

1) "The Retinoids," ed. by Sporn M. B., Roberts A. B., Goodman D. S., 2nd ed., Raven Press, New York, 1994.
2) Germain P., Chambon P., Eichele G., Evans R. M., Lazar M. A., Leid M., De Lera A. R., Lotan R., Mangelsdorf D. J., Gronemeyer H., *Pharmacol. Rev.*, **58**, 712–725 (2006).
3) Germain P., Chambon P., Eichele G., Evans R. M., Lazar M. A., Leid M., De Lera A. R., Lotan R., Mangelsdorf D. J., Gronemeyer H., *Pharmacol. Rev.*, **58**, 760–772 (2006).
4) Dawson M. I., Xia Z., *Biochim. Biophys. Acta*, **1821**, 21–56 (2012).
5) Pérez E., Bourguet W., Gronemeyer H., de Lera A. R., *Biochim. Biophys. Acta*, **1821**, 57–69 (2012).
6) Amano Y., Noguchi M., Nakagomi M., Muratake H., Fukasawa H., Shudo K., *Bioorg. Med. Chem.*, **21**, 4342–4350 (2013).
7) Cramer P. E., Cirrito J. R., Wesson D. W., Lee C. Y., Karlo J. C., Zinn A. E., Casali B. T., Restivo J. L., Goebel W. D., James M. J., Brunden K. R., Wilson D. A., Landreth G. E., *Science*, **335**, 1503–1506 (2012).
8) Shudo K., Fukasawa H., Nakagomi M., Yamagata N., *Curr. Alzheimer Res.*, **6**, 302–311 (2009).
9) Lee H. P., Casadesus G., Zhu X., Lee H. G., Perry G., Smith M. A., Gustaw-Rothenberg K., Lerner A., *Expert Rev. Neurother.*, **9**, 1615–1621 (2009).
10) Fukasawa H., Nakagomi M., Yamagata N., Katsuki H., Kawahara K., Kitaoka K., Miki T., Shudo K., *Biol. Pharm. Bull.*, **35**, 1206–1212 (2012).
11) Ohta K., Kawachi E., Inoue N., Fukasawa H., Hashimoto Y., Itai A., Kagechika H., *Chem. Pharm. Bull.*, **48**, 1504–1513 (2000).
12) Ohta K., Tsuji M., Kawachi E., Fukasawa H., Hashimoto Y., Shudo K., Kagechika H., *Biol. Pharm. Bull.*, **21**, 544–546 (1998).