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Reduction of Lipophilicity at the Lipophilic Domain of RXR Agonists Enables Production of Subtype Preference: RXRα-Preferential Agonist Possessing a Sulfonamide Moiety

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*Retinoid X receptor agonists (RXR agonists, rexinoids) are interesting candidates for the treatment of cancers such as tamoxifen-resistant breast cancer and taxol-resistant lung cancer. However, well-known RXR agonists possess a strong lipophilic character. In addition, although RXR has three subtypes, no subtype-selective RXR agonists are known. Thus we aimed to produce less-lipophilic and subtype-selective RXR agonists. By designing sulfonamide-type RXR agonists, 4-[*N*-methanesulfonyl-*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)amino]benzoic acid (**8a**) was found to prefer RXRα over RXRβ and RXRγ, although the potency is less than the potencies of well-known RXR pan-agonists. Moreover, our results suggest that the reduction of lipophilicity at the hydrophobic interaction region of RXR agonists enables production of RXR subtype preference. Our finding will be useful for the creation of more potent and less-lipophilic subtype-selective RXR agonists aimed at the reduction of undesirable side effects.*

Introduction

Nuclear receptors, which are derived from a common primordial gene, are ligand-dependent transcription factors.[1,2] There are 48 distinct nuclear receptors in humans which are classified into two types. One type comprises the receptors with known endogenous ligands, such as retinoic acid, vitamin D, thyroid hormone, steroid hormones, and/or lipids. The other type are orphan receptors whose functions have not been determined.[1,3] Nuclear receptors work as monomers or dimers by themselves or with other partners. Representative nuclear receptors that function as homodimers or heterodimers are retinoid X receptors (RXRs), whose endogenous ligands are 9-*cis*-retinoic acid (9-*cis*RA; **1**)[4] and docosahexaenoic acid (DHA; **2**)[5] (Figure 1).

The heterodimeric partners of RXRs contain retinoic acid receptors (RARs) that regulate cell differentiation and proliferation, vitamin D receptor (VDR) associated with bone metabolism, peroxisome proliferator-activated receptors (PPARs) associated with lipid metabolism, thyroid hormone receptors (TRs) involved with basal metabolites, and pregnane X receptors

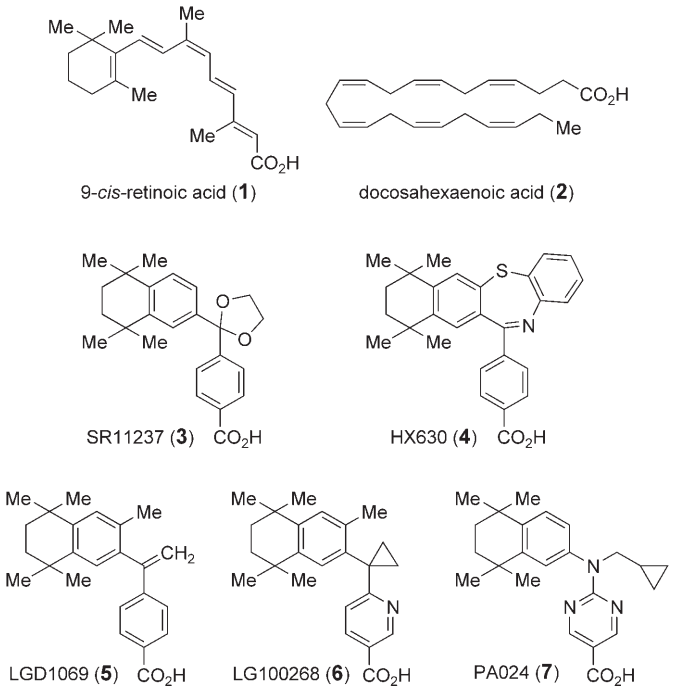

Figure 1. Chemical structures of known endogenous (**1**, **2**) and synthetic (**3**–**7**) RXR agonists.

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(PXRs) associated with expression of CYP3A4 involved in drug metabolism.[1] Therefore, RXRs are closely linked to the function of their partners, and RXR modulators enable control of the function of RXR heterodimeric partners.[6]

RXR agonists can act synergistically with partners. For example, when RXR agonists are treated with RAR agonists (so-called "retinoids", which are known as cell differentiation inducers), cell differentiation occurs typically at subefficacious concentration of RAR agonists alone.[7,8] Similar synergistic activities of RXR agonists are expected for those of other RXR heterodimeric partners such as VDR or PPARs.[6] Some RXR agonists (for example, LGD1069) have recently been used in clinical trials for the treatment of cancers such as tamoxifen-resistant breast cancer[9] and taxol-resistant lung cancer.[10] However, such RXR agonists possess a strong lipophilic character (Figure 1 and Table 1). A problem for clinical application would, therefore, be the possibility of undesirable side effects caused by the high lipophilicity. To prevent this problem, we decided to try to produce new RXR agonists whose lipophilicity are diminished.

Table 1. Calculated log *P* (Clog *P*) values of known RXR agonists.

Compd.:		3	4	5	6	7
Clog *P*	[a]	6.45	9.22	8.23	7.44	7.23
	[b]	5.92	8.31	6.81	5.37	5.02

[a] Calculated with ChemDraw Ultra 7.0. [b] Calculated with molinspiration (http://www.molinspiration.com)

RXR possess three different subtypes, RXRα, RXRβ, and RXRγ. RXRα is expressed mainly in the liver, kidney, and spleen, RXRβ is ubiquitously distributed, and RXRγ is expressed mainly in skeletal muscles, heart muscle, skin, and brain.[11] Nonetheless, no subtype-selective RXR agonists are known.[12,13] These facts encouraged us to develop less-lipophilic and subtype-selective RXR agonists. In this article, molecular design, synthesis, and bioactive assay of our new RXR agonists are reported.

Results and Discussion

To develop subtype-selective RXR agonists, we examined the information available. There is no apparent difference between the amino acid sequences in the ligand-binding domain of each RXR subtype.[14] Thus, activity and potency of various known RXR agonists for each subtype was examined in detail. PA024 (**7**) tends to act more potently toward RXRα/γ than RXRβ, which was not discussed in the previous report.[15] A comparison of the differences between characteristics of PA024 (**7**) and those of other known RXR agonists revealed that the Clog *P* value (a lipophilicity index) of PA024 (**7**) was lower than that of other compounds (Table 1). Highly lipophilic molecules tend to bind proteins nonspecifically. Thus, we hypothesized that less-lipophilic RXR agonists may acquire subtype specificity. This approach was judged to make it possible to reach our dual-purpose.

For designing less-lipophilic RXR agonists, we focused on a sulfonamide group, which possesses a polar character and is seen in many drugs, for example, sulfa drugs. Hence, a sulfonamide group was introduced into the "linking group" that connects the "acidic domain" and the "hydrophobic domain" of the generic RXR pharmacophore described in Figure 2.

Synthesis was performed as described in Scheme 1. After the preparation of tetrahydrotetramethylnaphthylamine (**11**) according to the reported method,[7] compounds **8a–d**, whose acidic domain is a benzoic acid, were synthesized by coupling reaction with ethyl 4-iodobenzoate using $Pd_2(dba)_3$ as a catalyst. After treatment with NaH in anhydrous DMF, the reaction mixture was treated with the corresponding alkylsulfonyl chloride to give sulfonamide ester intermediates. Deprotection of these esters was performed in an alkaline condition to afford the objective compounds **8a–d**.

Compound **9**, whose acidic domain is a nicotinic acid, was synthesized via the amino intermediate **13** by coupling reaction of amine (**11**) with 6-chloronicotinic acid in acetic acid under reflux. Compound **10**, whose acidic domain is a pyrimidine-5-carboxylic acid, was prepared via the amino intermediate **14** according to the reported method.[15,16] Then sulfonamidation and ester deprotection of compounds **13** and **14** was performed by the same procedure as that used for compounds **8a–d** to afford the objective compounds **9** and **10**, respectively.

RXR agonists, when combined with RAR agonists, induce cell differentiation in a synergistic fashion compared to RXR or RAR agonists (for example, Am80[7]) used alone. Cell differentiation can be observed using nitro blue tetrazolium (NBT) reduction.[13,17,18] We have tested compounds **8a–d**, **9**, and **10** for their ability to induce cell differentiation as a single agent or in combination with Am80. Table 2 shows retinoid or retinoid synergistic activities of our compounds and PA024 (**7**). None of the compounds **8a–d**, **9**, and **10** exhibited retinoid activity, suggesting they are not RAR agonists. A marked synergistic activity was observed with **8a**, **9**, and **10**, however with a reduced potency and efficacy compared to PA024 (**7**). Interest-

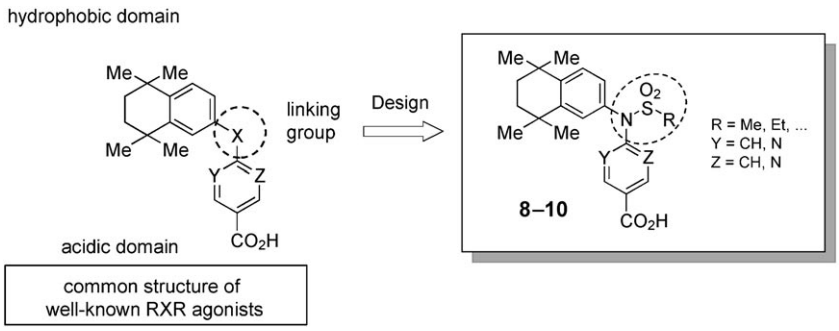
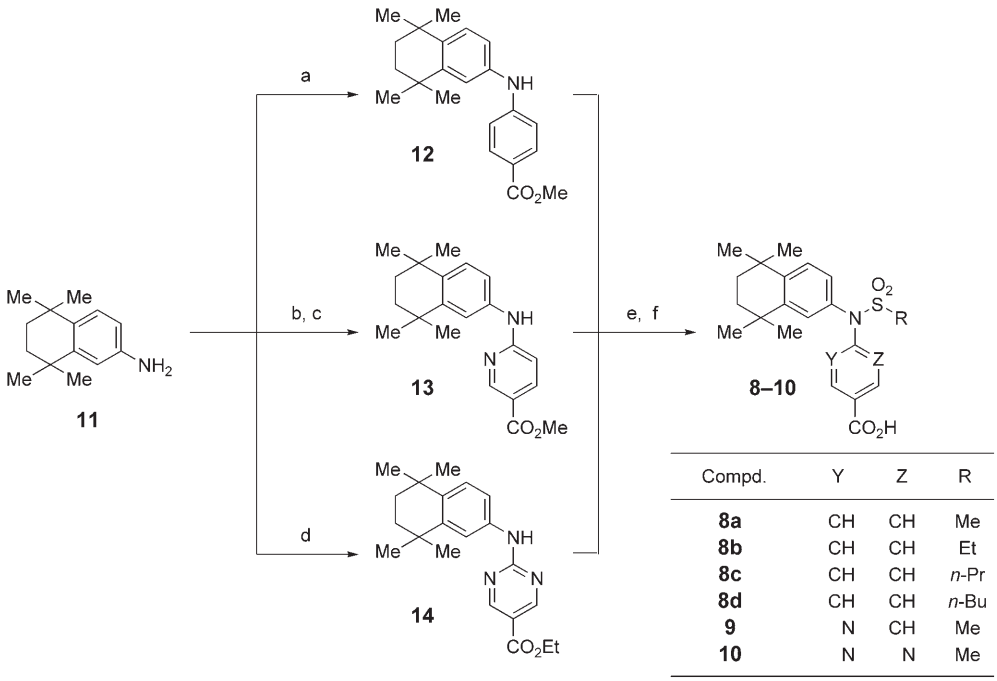


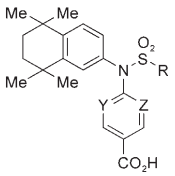
Figure 2. Strategy for the molecular design of low-lipophilic RXR agonists possessing sulfonamide moieties (**8**–**10**).



Compd.	Y	Z	R
8a	CH	CH	Me
8b	CH	CH	Et
8c	CH	CH	*n*-Pr
8d	CH	CH	*n*-Bu
9	N	CH	Me
10	N	N	Me

Scheme 1. Reagents and conditions: a) methyl-4-iodobenzoate, BINAP, $Pd_2(dba)_3$, Cs_2CO_3, toluene; b) 6-chloronicotinic acid, AcOH; c) MeOH, H_2SO_4; d) ethyl-2-chloro-5-pyrimidinecarboxylate, K_2CO_3, DMF; e) RSO_2Cl, NaH, DMF; f) LiOH, H_2O, THF.

Table 2. Cell differentiation inducing activity of compounds **8**–**10**, determined by NBT reduction assay.[a]



Compd.	R	Y	Z	Retinoid activity		Retinoid synergist activity	
				EC_{50}[b] [nM]	BA[c] [%]	EC_{50}[b] [nM]	BA[c] [%]
8a	Me	CH	CH	>10000	2±0	300±30	67±5
8b	Et	CH	CH	Inactive	–	830±10	42±0
8c	*n*-Pr	CH	CH	Inactive	–	Inactive	–
8d	*n*-Bu	CH	CH	Inactive	–	Inactive	–
9	Me	N	CH	>10000	4±1	150±10	73±1
10	Me	N	N	>10000	1±0	2200±200	56±0
PA024 (**7**)				Inactive	–	3.3±0.6	83±1

[a] All values were determined from full dose-response curves ranging from 10^{-9} to 10^{-5} M with HL-60 cells. Where errors are indicated, values represent the standard error of the mean value of at least two separate experiments. [b] EC_{50} was determined as the concentration of a test compound that was required to elicit a response at half-maximal height on the dose-response curve. [c] Biological activity (%) is maximal differentiation ratio that was induced by a test compound.

ingly, not showing retinoid synergistic activity, compounds **8c** and **8d** indicated a tendency to inhibit compound **9**-induced-retinoid synergistic activity (data not shown).

Next, these compounds were assayed for luciferase transcription activities by a reporter gene assay[15,19,20] to compare their potencies toward each RXR subtype. As SR11237[21] (**3**) possesses polar cyclic acetal moiety, the Clog*P* value was thought to be low and show subtype-preference (activity toward RXRα was only reported). Table 3 shows EC_{50} and E_{max} values in each RXR subtype and Clog*P* values of compounds **8a**–**d**, **9**, **10**, SR11237 (**3**), LGD1069[22] (**5**), and PA024 (**7**). All tested compounds showed transcription activities, indicating that their retinoid synergistic activities were mediated by RXR. Compounds **8b**–**d** showed weak RXR agonist activities, as expected from the results of the NBT assay. Notably, compounds **8a**, **9**, and SR11237 (**3**) showed lower Clog*P* values than LGD1069 (**5**) and an apparent difference in EC_{50} between each subtype. The ratio of EC_{50} values between RXRα:RXRβ:RXRγ for LGD1069 (**5**) was 1.0:2.0:1.6, in contrast, that for PA024 (**7**) was 1.0:8.0:2.6. SR11237 (**3**) shows that the ratio of EC_{50} values between RXRα:RXRβ:RXRγ was 1.0:3.3:9.0. For compound **8a**, the ratio of EC_{50} values between RXRα:RXRβ:RXRγ was 1.0:11.5:3.1, indicating that this compound prefers RXRα/γ over RXRβ. Considering the fact that the E_{max} value of **8a** toward RXRα is larger than that of RXRβ or RXRγ by 50%, compound **8a** can be regarded as an RXRα-preferential agonist. SR11237 (**3**), PA024 (**7**), compounds **8a**, and **9** show lower Clog*P* values than LGD1069 (**5**) and subtype-preference, supporting our hypothesis that less-lipophilic RXR agonists are able to produce RXR subtype selectivity. In contrast, compound **10**, whose Clog*P* value is the lowest in this study,

Table 3. Co-transfection data for compounds **8**–**10** and known RXR agonists (**3**, **5**, and **7**).[a]

Compd.	RXRα		RXRβ		RXRγ		Clog *P*[d]
	EC_{50}[b] [nM]	E_{max}[c] [%]	EC_{50}[b] [nM]	E_{max}[c] [%]	EC_{50}[b] [nM]	E_{max}[c] [%]	
8a	195±25	115±16	2250±0	52±14	620±50	59±3	6.55
8b	307±1	79±1	2009±579	102±4	1254±431	112±3	7.08
8c	2019±203	71±7	1452±452	24±11	2347±530	73±32	7.61
8d	2795±112	33±10	2201±868	28±11	2397±239	17±4	8.14
9	115±5	98±6	635±75	94±2	350±85	81±7	6.17
10	1888±488	147±7	1995±394	84±6	1519±130	42±6	5.46
SR11237 (**3**)	29±3	111±8	98±27	106±7	232±82	122±4	6.45
LGD1069 (**5**)	3±0	106±12	6±1	114±12	5±2	104±3	8.23
PA024 (**7**)	3±1	100	24±0	100	8±1	100	7.23

[a] All values represent the standard error of the mean value of at least two separate experiments with triplicate determinations. [b] EC_{50} values were determined from full dose-response curves ranging from 10^{-9} to 10^{-5} M in COS-1 cells. [c] Luciferase activity of PA024 (**7**) at 1 μM was defined as 100%. [d] Clog *P* values were calculated with ChemDraw Ultra 7.0.

showed lower subtype-preference and potency than compound **8a**. Considering this result, we are proposing that production of subtype-preference, reduction of lipophilicity should be performed at the lipophilic domain of RXR agonists rather than at the acidic domain.

To understand the reason why compound **8a** shows subtype preference, a docking study was performed with AutoDock.[23] Unfortunately, no apparent differences in amino acid sequences and positions in the ligand-binding domain in each RXR subtype were found (data not shown). It seems difficult to discuss the difference by docking simulation visually. The results shown in Figure 3, however, revealed a promising binding affinity of compound **8a**, which was docked into the binding site of 1mvc RXR receptor exactly superimposed on the native bound ligand (BMS649: the same as SR11237 (**3**)) with root of mean square deviation (RMSD) of 0.604 Å. Moreover, compound **8a** formed three hydrogen bonds with the same atoms of amino acids Arg^{316} and Ala^{327} as the native ligand, SR11237 (**3**). It should be noticed that the methanesulfonamide moiety is deeply embedded into the lipophilic residue including Leu^{309}, Ile^{310}, and Cys^{432} within distances of 2.99, 2.61, and 3.21 Å, respectively. This close interaction may explain the improper fitting of compound **8a** into the RXR owing to its methanesulfonamide moiety. These results indicate that reduction of molecular lipophilicity is required to produce RXR subtype selectivity and that the introduction of a polar moiety into the linking group should be avoided for the creation of more potent subtype-selective RXR agonists.

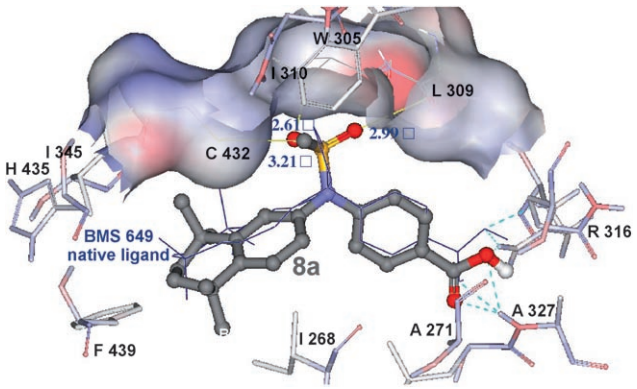


Figure 3. Docking model of compound **8a** (ball and stick, colored by element) in the binding site of RXR (PDB code: 1mvc) using AutoDock3.05. It exhibited 3 H-bonds between Arg^{316} and Ala^{327} (dotted blue lines) with RMSD of 0.604 Å from BMS649 (the same as SR11237 (**3**)), the native ligand.

Several RXR agonists have recently been used in the clinical trial as therapeutic agents against cancer or as chemopreventive agents.[12] Other RXR agonists have also been described as insulin sensitizer or anti obese agents and were shown to decrease blood sugar or weight gain in rodent models.[24,25] Therefore, less lipophilic and subtype-selective RXR agonists we are seeking for are expected to be therapeutic agents for cancer or metabolic syndrome, and may be useful biological tools for elucidating each RXR subtype function.

Conclusions

In this study, we aimed to produce less lipophilic and subtype-selective RXR agonists. By designing sulfonamide-type RXR agonists, 4-[*N*-methanesulfonyl-*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)amino]benzoic acid (**8a**) was found to prefer RXRα over RXRβ and RXRγ, although the potency is less than the potencies of well-known RXR pan-agonists. Moreover, our results suggest that for production of subtype-preferential RXR agonists, reduction of lipophilicity should be performed at the lipophilic domain of RXR agonists rather than at the acidic domain. In the future, modification of our strategy should enable production of more potent, subtype-selective RXR agonists, which may be useful for elucidation of each RXR subtype function and/or development of RXR drugs with appropriate action spectra.

Experimental Section

Chemistry

Melting points were determined with a Yanagimoto hot-stage melting point apparatus and are uncorrected. IR were recorded on JASCO FT/IR350 (KBr). ^{1}H NMR spectra were recorded on a Var-

ianVXR-300 (300 MHz) or VarianVXR-500 (500 MHz) spectrometer. Elemental analysis was carried out with a Yanagimoto MT-5 CHN recorder elemental analyzer. FAB-MS was carried out with a VG70-SE.

SR11237 (3) and LGD1069 (5). These compounds were prepared according to references [26] and [22], respectively.

5,5,8,8-Tetramethyl-5,6,7,8-tetrahydro-2-naphthylamine (11). This compound was prepared according to reference [7].

Methyl-*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)-4-aminobenzoate (12). Concentrated H_2SO_4 (1.5 mL) on cooling was added to a solution of 4-iodobenzoic acid (5.0 g, 20 mmol) in dry MeOH (50 mL), and the mixture was held at reflux for 4 h. The reaction mixture was concentrated under reduced pressure and $NaHCO_3$ solution (300 mL) was added to the residue. The mixture was extracted with EtOAc (3×200 mL). After being washed with H_2O (200 mL) and brine (200 mL), the organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to yield methyl 4-iodobenzoate. Recrystallization from CH_2Cl_2 afforded colorless cubes (72–85%); mp: 105.0–106.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.81 (2H, d, J = 8.5 Hz), 7.75 (2H, d, J = 8.5 Hz), 3.91 ppm (3H, s); IR (KBr): $\tilde{\nu}$ = 1714 cm^{-1}.

Dry toluene (20.0 mL) was added to a mixture of methyl 4-iodobenzoate (2.62 g, 10 mmol), amine **11** (2.03 g, 10 mmol), (±)-BINAP (0.47 g, 0.75 mmol), $Pd_2(dba)_3$ (0.46 g, 0.5 mmol), and Cs_2CO_3 (4.56 g, 14.0 mmol) under argon at room temperature. The mixture was held at reflux for 16 h. The slurry was filtered through Celite, and the Celite cake was washed with EtOAc (500 mL). After being washed with H_2O (2×150 mL) and brine (150 mL), the organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 8:1) to afford compound **12**. Recrystallization from CH_2Cl_2/*n*-hexane afforded colorless cubes (85%); mp: > 200 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.90 (2H, d, J = 8.5 Hz), 7.27 (1H, d, J = 8.5 Hz), 7.09 (1H, s), 6.96 (1H, d, J = 8.5 Hz), 6.93 (2H, d, J = 9.0 Hz), 3.87 (3H, s), 1.69 (4H, s), 1.28 (6H, s) 1.27 ppm (6H, s); IR (KBr): $\tilde{\nu}$ = 3357, 1693 cm^{-1}.

Methyl-[*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)]-6-aminonicotinate (13). Compound **11** (610 mg, 3.0 mmol) was added to a solution of 6-chloronicotinic acid (473 mg, 3.0 mmol) in AcOH (3.0 mL), and the mixture was held at reflux for 10 h. The reaction mixture was poured into H_2O (100 mL) and extracted with EtOAc (3×100 mL). After being washed with H_2O (100 mL), the organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. Recrystallization from EtOAc afforded [*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)]-6-aminonicotinic acid as colorless cubes (633 mg, 60%). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.55 (1H, br s), 9.40 (1H, s), 8.64 (1H, d, J = 2.5 Hz), 7.94 (1H, dd, J = 9.0, 2.5 Hz), 7.52 (1H, dd, J = 8.5, 2.5 Hz), 7.43 (1H, d, J = 2.5 Hz), 7.25 (1H, d, J = 8.5 Hz), 6.79 (1H, d, J = 9.0 Hz), 1.64 (4H, s), 1.25 (6H, s), 1.23 ppm (6H, s); FAB-MS *m/z*; 325 [*M*+H]$^+$.

Concentrated H_2SO_4 (0.07 mL) on cooling was added to a solution of [*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)]-6-aminonicotinic acid (150 mg, 0.6 mmol) in dry MeOH (1.5 mL) and the mixture was held at reflux overnight. The reaction mixture was concentrated under reduced pressure and poured into a sat. $NaHCO_3$ solution (100 mL). The mixture was extracted with EtOAc (2×100 mL). After being washed with H_2O (100 mL) and brine (100 mL), the organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to yield compound **13** (123 mg, 80%). As this compound gave a single spot on TLC, **13** was used for the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.79 (1H, d, J = 2.5 Hz), 8.04 (1H, dd, J = 9.0, 2.5 Hz), 7.37 (1H, s), 7.32 (1H, d, J = 8.5 Hz), 7.20 (1H, d, J = 2.5 Hz), 7.10 (1H, dd, J = 8.5, 2.5 Hz), 6.80 (1H, d, J = 9.0 Hz), 3.89 (3H, s), 1.70 (4H, s), 1.29 (6H, s), 1.28 ppm (6H, s); FAB-MS *m/z*; 339 [*M*+H]$^+$.

Ethyl-[*N*-(2,5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)]-2-amino-pyrimidine carboxylate (14). This compound was prepared according to references [15] and [16].

General procedure for the synthesis of *N*-alkylsulfonamide intermediates. The corresponding intermediates (**12**–**14**) (1.0 mmol) were added to a DMF solution (5.0 mL) containing sodium hydride (2.5 mmol, 60% purity in oil) under argon at room temperature for 5 min. Then, a corresponding alkylsulfonyl chloride (1.2 mmol) was added to the solution. After stirring for 2 h, the mixture was poured into H_2O, and extracted with EtOAc. The organic phase was washed with H_2O and brine and then dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography to afford *N*-alkylsulfonamide intermediates.

General procedure for the synthesis of [*N*-alkylsulfonyl-*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)]-4-aminobenzoic acids (8a–d). LiOH·H_2O (2.0 mmol) was added to a solution of *N*-alkylsulfonamide intermediates (1.0 mmol) in THF/H_2O (2.0 mL, 3:1) and the mixture was kept at room temperature over night. The mixture was poured into 2 N HCl (15.0 mL) and extracted with EtOAc (3×20 mL). After being washed with H_2O (30 mL) and brine (30 mL), the organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. Recrystallization gave the target molecules **8a**–**d**.

[*N*-Methanesulfonyl-*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)]-4-aminobenzoic acid (8a). Colorless needles from EtOAc/*n*-hexane; mp: 165.0–166.0 °C; Yield 71%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.98 (1H, br s), 7.94 (2H, d, J = 8.5 Hz), 7.44 (1H, dd, J = 8.5, 2.5 Hz), 7.40 (1H, d, J = 8.5 Hz), 7.37 (1H, d, J = 2.5 Hz), 7.18 (2H, d, J = 8.5 Hz), 3.33 (3H, s), 1.64 (4H, s), 1.25 (6H, s), 1.22 ppm (6H, s); IR (KBr): $\tilde{\nu}$ = 2962–2800, 1687 cm^{-1}; FAB-MS *m/z*; 401 [*M*]$^+$, 402 [*M*+H]$^+$; Anal. Calcd for $C_{22}H_{27}NO_4S$: C, 65.81; H, 6.78; N, 3.49. Found: C, 65.77; H, 6.61; N, 3.41.

[*N*-Ethanesulfonyl-*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)]-4-aminobenzoic acid (8b). Colorless columns from CH_2Cl_2/*n*-hexane; mp: 167.0–168.0 °C; Yield 43%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.00 (1H, br s), 7.92 (2H, d, J = 9.0 Hz), 7.44 (2H, d, J = 9.0 Hz), 7.42 (1H, d, J = 8.5 Hz), 7.36 (1H, d, J = 2.5 Hz), 7.18 (1H, dd, J = 8.5, 2.5 Hz), 3.42 (2H, q, J = 7.5 Hz), 1.64 (4H, s), 1.31 (3H, t, J = 7.5 Hz), 1.25 (6H, s), 1.22 ppm (6H, s); IR (KBr): $\tilde{\nu}$ = 1719, 1335, 1139 cm^{-1}; FAB-MS *m/z*; 415 [*M*]$^+$, 416 [*M*+H]$^+$; Anal. Calcd for $C_{23}H_{29}NO_4S$: C, 66.48; H, 7.03; N, 3.37. Found: C, 66.28; H, 6.97; N, 3.46.

[*N*-*n*-Propanesulfonyl-*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)]-4-aminobenzoic acid (8c). Colorless needles from CH_2Cl_2/*n*-hexane; mp: 201.0–203.0 °C; Yield 32%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.94 (1H, br s), 7.92 (2H, d, J = 9.0 Hz), 7.44 (2H, d, J = 9.0 Hz), 7.39 (1H, d, J = 8.5 Hz), 7.35 (1H, d, J = 2.0 Hz), 7.18 (1H, dd, J = 8.5, 2.0 Hz), 3.39 (2H, t, J = 7.5 Hz), 1.79 (2H, sex, J = 7.5 Hz), 1.64 (4H, s), 1.24 (6H, s) 1.22 (6H, s), 1.00 ppm (3H, t, J = 7.5 Hz); IR (KBr): $\tilde{\nu}$ = 1688, 1329, 1148 cm^{-1}; FAB-MS *m/z*; 430 [*M*+H$^+$]; Anal. Calcd for $C_{24}H_{31}NO_4S$: C, 67.01; H, 7.27; N, 3.26. Found: C, 67.00; H, 7.20; N, 3.44.

[*N*-*n*-Butanesulfonyl-*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)]-4-aminobenzoic acid (8d). White needles from CH_2Cl_2/

n-hexane; mp: 213.0–215.0 °C; Yield 38%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.01 (1H, br s), 7.92 (2H, d, J = 9.0 Hz), 7.44 (2H, d, J = 9.0 Hz), 7.39 (1H, d, J = 8.5 Hz), 7.35 (1H, d, J = 2.0 Hz), 7.18 (1H, dd, J = 8.5, 2.0 Hz), 3.42 (2H, t, J = 7.5 Hz), 1.73 (2H, sex, J = 7.5 Hz), 1.64 (4H, s), 1.42 (2H, m), 1.24 (6H, s), 1.22 (6H, s), 0.88 ppm (3H, t, J = 7.5 Hz); IR (KBr): $\tilde{\nu}$ = 1688, 1332, 1173 cm^{-1}; FAB-MS *m/z*; 443 [*M*]$^+$, 444 [*M*+H]$^+$; Anal. Calcd for $C_{25}H_{33}NO_4S$: C, 67.69; H, 7.50; N, 3.16. Found: C, 67.35; H, 7.31; N, 3.12.

[*N*-Methanesulfonyl-*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)]-6-aminonicotinic acid (9). Colorless needles from CH_2Cl_2/*n*-hexane; mp: 228.0–228.5 °C; Yield 89%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.89 (1H, d, J = 2.5 Hz), 8.16 (1H, dd, J = 8.5, 2.5 Hz), 7.45 (2H, d, J = 8,5 Hz), 7.33 (1H, d, J = 2.5 Hz), 7.12 (1H, dd, J = 8.5 Hz, 2.5 Hz), 6.63 (1H, d, J = 8.5 Hz), 3.59 (3H, s), 1.67 (4H, s), 1.28 (6H, s), 1.23 ppm (6H, s); IR (KBr): $\tilde{\nu}$ = 1690, 1366, 1171 cm^{-1}; FAB-MS *m/z*; 403 [*M*+H]$^+$; Anal. Calcd for $C_{21}H_{26}N_2O_4S$: C, 62.66; H, 6.51; N, 6.96. Found: C, 62.62; H, 6.29; N, 6.55.

[*N*-Methanesulfonyl-*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)]-2-amino-pyrimidine carboxylic acid (10). White cubes from EtOAc/*n*-hexane; mp: 248.0–250.0 °C; Yield 87%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.13 (2H, s), 7.39 (1H, d, J = 8,5 Hz), 7.17 (1H, d, J = 2.5 Hz), 7.04 (1H, dd, J = 8.5 Hz, 2.5 Hz), 3.63 (3H, s), 1.71 (4H, s), 1.31 (6H, s), 1.27 ppm (6H, s); IR (KBr): $\tilde{\nu}$ = 1696, 1369, 1173 cm^{-1}; FAB-MS *m/z*; 404 [*M*+H]$^+$; Anal. Calcd for $C_{20}H_{25}N_3O_4S$: C, 59.53; H, 6.25; N, 10.41. Found: C, 59.52; H, 6.33; N, 10.53.

Calculation of Clog*P* values. Log*P* values for compounds were calculated with ChemDraw Ultra 7.0 or software available from molinspiration (http://www.molinspiration.com/).

NBT reduction assay

Culture of HL-60 cells. The human promyelocyte leukemia cell line HL-60 was cultured in RPMI1640, which contained 10% fetal bovine serum (FBS) and antibiotics (2% of penicillin-streptomycin solution purchased from SIGMA), in a humidified atmosphere of 5% CO_2 at 37 °C.

NBT reduction assay.[13,17,18] Test compounds were dissolved in DMSO at 20 mM for stock solutions. To a suspension of cells at a concentration of 8×10^4 cells mL^{-1} was added a test compound solution in DMSO. Final DMSO concentration was kept below 0.1%. For a vehicle and a positive control, the same volume of DMSO and Am80[7] solution in DMSO were added, respectively. After incubation for 4 days, NBT reduction assay was performed as described below. Cells were incubated in RPMI1640 (10% FBS) and an equal volume of phosphate-buffer saline (PBS (−)) containing 0.2 *w/w*% NBT and 12-*O*-tetradecanoylphorbol-13-acetate (TPA, 200 ng mL^{-1}) in a humidified atmosphere of 5% CO_2 at 37 °C for 30 min. The rate of cell differentiation was calculated by the percentage of cells containing blue–black formazan using more than 200 cells. Average of at least three results for each assay was calculated. Synergistic activities of test compounds with Am80 were evaluated in the presence of 3.3×10^{-10} M of Am80, which induces less than 10% of cell differentiation, according to the method described above.

Luciferase reporter gene assay

Culture of COS-1 cells. COS-1 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS in a humidified atmosphere of 5% CO_2 at 37 °C.

Luciferase reporter gene assay.[15,19,20] Luciferase reporter gene assays were performed using COS-1 cells transfected with three kinds of vectors; each RXR subtype, a luciferase reporter gene under the control of the appropriate RXR response elements, and secreted alkaline phosphatase (SEAP) gene as a background. A CRBPII-tk-Luc reporter and plasmid DNAs were purified by a QIA filter Plasmid Midi kit. COS-1 cells were transfected with QIA Effectene Transfection reagent according to the supplier's protocol. Test compound solutions whose DMSO concentrations were below 1% were added to the suspension of transfected cells, which were seeded at about 2×10^5 cells mL^{-1} in 96-well white plates. For vehicle and positive control, the same volume of DMSO and 9-*cis*RA solution in DMSO were added, respectively. After incubation in a humidified atmosphere of 5% CO_2 at 37 °C for 18 h, the parts of the medium were used for SEAP and the remaining cells were used for luciferase reporter gene assays with a Steady-Glo Luciferase Assay system (Promega) according to the supplier's protocol. The luciferase activities were normalized using secreted alkaline phosphatase (SEAP) activities. The assays were carried out in duplicate three times.

Molecular docking

The crystal structure of the human RXRα–ligand binding domain (PDB code: 1mvc) was retrieved from the Brookhaven Protein Data Bank: http://www.rcsb.org/pdb/Wecome.do accessed in December 10, 2005. Polar hydrogen atoms were added to both the protein and the ligand. United atom Kollman charges were assigned for the protein. The 3D structures of ligands used for the docking study were constructed by using Chem3D Ultra 8.0 software [Molecular Modeling and Analysis; Cambridge Soft Corporation, USA (2003)]. These ligands were energetically minimized by using MOPAC (semi-empirical quantum mechanics) with AM1 MOZYME geometry. The AutoDock3.05 molecular docking program[23] was employed by using a genetic algorithm with local search (GALS). One hundred individual GA runs, 150 chromosomes, a crossover ratio of 0.80, a rate of gene mutation of 0.02, and an elitism ratio of 0.10 were used for each ligand. The grid box was created with dimensions of $60\times60\times60$ Å^3 which encloses the original ligand BMS649 (SR11237 (**3**)). The box spacing was 0.3 Å. Accelrys Discovery Studio version 1.6 [Accelrys inc., San Diego, CA (2006)] was used for molecular modeling, and the mode of interaction of BMS649 (SR11237 (**3**)) against 1 mvc was used as a standard docked model as well as for RMSD calculation.

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