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The First Potent Subtype-Selective Retinoid X Receptor (RXR) Agonist Possessing a 3-Isopropoxy-4-isopropylphenylamino Moiety, NEt-3IP (RXRα/β-dual agonist)

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*Retinoid X receptor (RXR) agonists (rexinoids) are attracting much attention for their use in treatment of cancers, including tamoxifen-resistant breast cancer and taxol-resistant lung cancer, and metabolic disease. However, known RXR agonists have a highly lipophilic character. In addition, no subtype-selective RXR agonists have been found. We previously reported an RXRα-preferential agonist 4-[*N*-methanesulfonyl-N-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)amino]benzoic acid (**6a**). The RXR agonistic activity is much less than that of well-known RXR agonists. To develop potent, less-lipophilic, and subtype-selective RXR agonists, we created new RXR agonists possessing alkoxy and isopropyl groups as a lipophilic domain of the common structure of well-known RXR agonists. As a result, compounds possessing branched alkoxy groups, 6-[*N*-ethyl-N-(3-isopropoxy-4-isopropylphenyl)amino]nicotinic acid (NEt-3IP: **7a**) and 6-[*N*-ethyl-N-(3-isobutoxy-4-isopropylphenyl)amino]nicotinic acid (NEt-3IB: **7c**), showed RXR agonistic activity as potent as, or more potent than, the activities of representative RXR agonists. Moreover, NEt-3IP (**7a**) was found to be the first RXRα/β-selective (or RXRα/β-dual) agonist. Being potent, less lipophilic, and having RXR subtype-selective activity, NEt-3IP (**7a**) is expected to become a new drug candidate and to be a useful biological tool for clarifying each RXR subtype function.*

Introduction

Much interest has recently been shown in retinoid X receptors (RXRs) as targets in the treatment of cancers such as tamoxifen-resistant breast cancer and taxol-resistant lung cancer, and metabolic disease.[1–4] RXRs belong to the nuclear receptor superfamily of proteins that regulate many physiological functions in a ligand-dependent manner.[2,5] Nuclear receptors include retinoic acid receptors (RARs), which regulate cell differentiation and proliferation, vitamin D receptor (VDR) which is associated with bone metabolism, and peroxisome proliferator-activated receptors (PPARs), which are associated with lipid metabolism.[2,5,6] Nuclear receptors work as monomers or dimers by themselves or with other partners. Representative nuclear receptors forming heterodimers are RARs, VDR, and PPARs, which function with RXRs.[2,6] Therefore, RXRs are closely linked to the function of such partners, and RXR agonists control synergistically the function of RXR heterodimeric partners.[2,7] RXR agonists are also expected to be dose reducers for RXR heterodimer agonists that possess lipophilic characteristics and can have undesirable side effects owing to their accumulation in adipose tissue or liver.

RXRs have three subtypes (RXRα, β, and γ), which are coded by different genes and are distributed in different locations in the body.[1,2,8] 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. Figure 1 shows representative endogenous or synthetic RXR agonists. Endogenous RXR agonists (**1**, **2**) and synthetic RXR agonists (**3**–**5**) have a highly lipophilic character and lack of subtype selectivity. Actually, no subtype-selective RXR agonists have been found.[2,9,10] These backgrounds prompted us to create new RXR agonists with low lipophilicity and subtype selectivity.

We have found that reduction of lipophilicity seems to enable production of subtype-selective RXR agonists, and we have discovered a relative subtype-selective RXR agonist 4-[*N*-methanesulfonyl-*N*-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)amino]benzoic acid (**6a**), which prefers RXRα over

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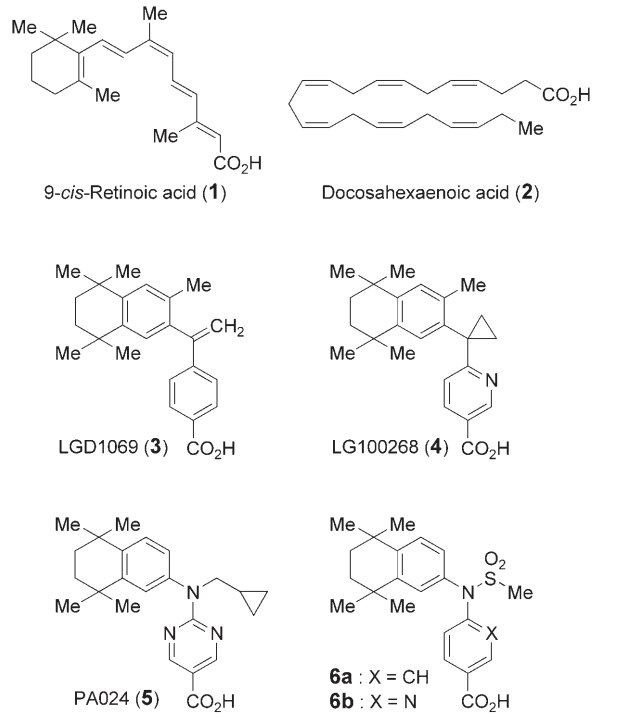


Figure 1. Chemical structures of known endogenous (**1**, **2**) or synthetic (**3**–**6**) RXR agonists.

RXRβ and RXRγ (Figure 1).[11] However, the RXR agonistic activity is much less than that of well-known RXR agonists. The reason for the weak activity is thought to be the introduction of a polar moiety such as a sulfonamide group into the so-called linking group of the common structure of well-known RXR agonists (Figure 2).[11] In this study, we aimed to develop potent, less-lipophilic and subtype-selective RXR agonists. As a result, we discovered 6-[*N*-ethyl-*N*-(3-isopropoxy-4-isopropylphenyl)amino]nicotinic acid (NEt-3IP: **7a**) as the first RXRα/β-selective (RXRα/β-dual) agonist. Herein we report the molecular design and in vitro evaluation.

Design strategy

Our previous results suggest that reduction of lipophilicity at the lipophilc domain of RXR agonists enables production of subtype preference.[11] To develop potent subtype-selective RXR agonists, we replaced a tetramethyl-cyclohexyl ring, the common hydrophobic domain of well-known RXR agonists, with alkoxy and isopropyl groups, which have more polar characteristics. As it was also found that potent RXR activity requires a lipophilic moiety on the linking amino group,[11] *N*-ethylation was performed for moderate lipophilicity. Nicotinic acid or pyrimidine carboxylic acid was applied to the acidic domain (Figure 2).

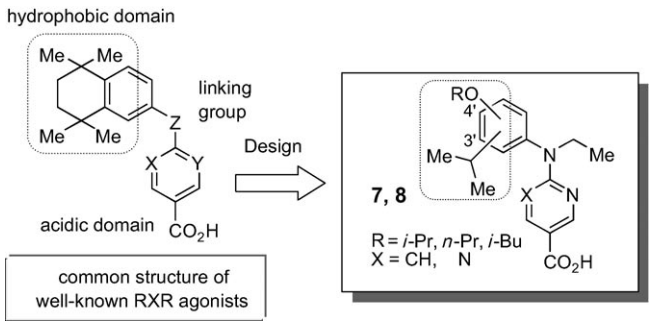


Figure 2. Strategy for the molecular design of subtype-selective RXR agonists possessing alkoxy and isopropyl groups (**7**, **8**).

Chemistry

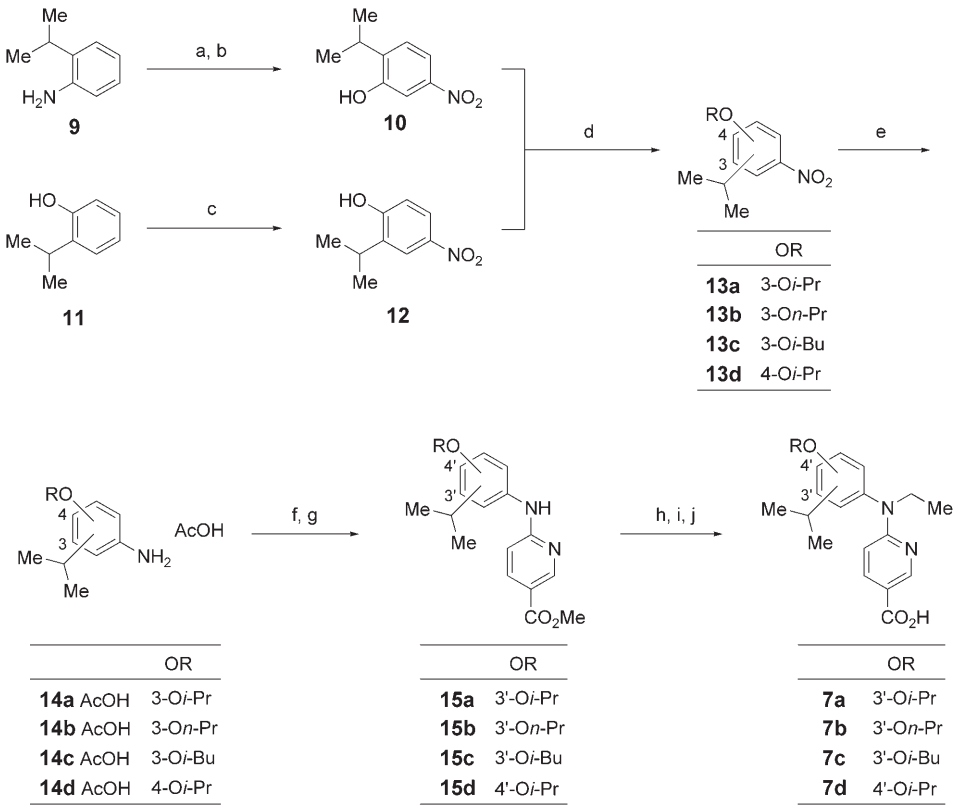
Synthesis was performed by the following steps: synthesis of anilino derivatives, coupling of them with chloronicotinic acid or chloropyrimidine carboxylic acid ester, *N*-ethylation of the linking amino group, and deprotection of esters. In Scheme 1, synthesis of nicotinic acid derivatives is shown. Anilino derivatives possessing an alkoxy group at the 3-position were prepared by the following procedure: nitration of 2-isopropylaniline with sulfuric acid and nitric acid, replacement of the amino group with a hydroxy group by Sandmyer reaction, *O*-alkylation with the corresponding alkyl halide, and hydrogen reduction. On the other hand, anilino derivatives possessing an alkoxy group at the 4-position were prepared from 2-isopropylphenol by radical nitration with nitric acid and zinc chloride in an ultrasonic reactor,[12] with similar alkylation and reduction to that described above. The anilino derivatives were reacted with 6-chloronicotinic acid in acetic acid, and the carboxyl groups of the products were protected as methyl ester. After *N*-ethylation of the linking amino group, deprotection of the methyl ester gave the objective compounds **7a**–**7d**.

Compounds **8a** and **8c**, whose acidic domains are a pyrimidine-5-carboxylic acid, were prepared via the amino intermediates hydrochloride **14a** HCl and **14c** HCl according to the reported method (Scheme 2).[13,15] Then *N*-alkylation and ester deprotection of compounds **16a** and **16c** were performed to afford the objective compounds **8a** and **8c**, respectively.

Results and Discussion

RXR agonists alone do not exhibit cell differentiation activity, although they work synergistically with RAR agonists (for example, Am80[10]) to differentiate the human promyelocyte leukemia cell line HL-60 cells to granulocytes.[10,15] This phenomenon is based on the synergistic action of RXR with RAR. For the first screening of the compounds, the activity of compounds alone (retinoid activity) and the activity of compounds with RAR agonists (retinoid synergistic activity) were examined with HL-60.[10,16,17] In this screening, cell differentiation activities were evaluated with nitro blue tetrazolium (NBT) reduction.

First, to examine the proper position of an alkoxy group on the phenylamino moiety, the retinoid and retinoid synergistic activities of NEt-3IP (**7a**) and NEt-4IP (**7d**) were assessed (Table 1). Neither of them showed retinoid activities, suggesting that they do not activate RAR directly. On the other hand, retinoid synergistic activities of **7a** and **7d** were more potent than or as potent as those of sulfonamide-type RXR agonists

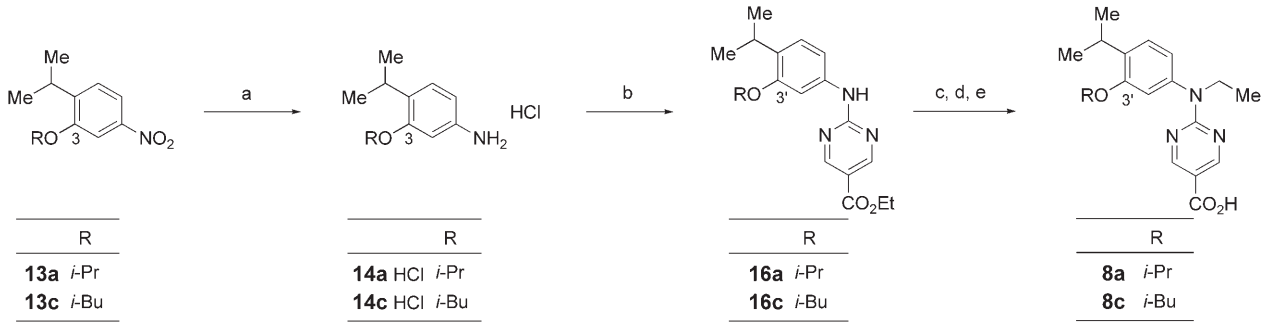


Scheme 1. Reagents and conditions: a) H_2SO_4, HNO_3. b) $NaNO_2$, H_2SO_4. c) HNO_3, EtOAc, $ZnCl_2$, u-sonic. d) RX, K_2CO_3, DMF. e) H_2, Pd-C, AcOH. f) 6-Chloronicotinic acid, AcOH. g) MeOH, H_2SO_4. h) EtI, NaH, DMF. i) NaOH, MeOH. j) HCl.

retinoid activity similar to that of NEt-3IP (**7a**). The retinoid synergistic activity of NEt-3NP (**7b**) possessing a linear alkoxy group, *n*-propoxy, was less potent than that of NEt-3IP (**7a**) and NEt-3IB (**7c**), suggesting that branched alkoxy groups are appropriate for potent retinoid synergistic activities.

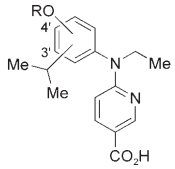
Next, to identify those retinoid synergistic activities exerted via RXR and to compare their potencies toward each RXR subtype, luciferase transcription activities by a reporter gene assay were evaluated.[13,18,19] Table 2 shows their EC_{50} and E_{max} values for each RXR subtype. All of the compounds showed transcription activities, indicating that their retinoid synergistic activities were mediated by RXR. As with the results of the NBT reduction assay, transcription activities of NEt-3IP (**7a**) were found to be more potent than those of NEt-4IP (**7d**). Notably, NEt-3IP (**7a**) and NEt-3IB (**7c**) showed an apparent difference in EC_{50} between each subtype. For NEt-3IP (**7a**), the ratio of EC_{50} (mean values) of RXRα and RXRβ to that of RXRγ was more than tenfold, indicating that this compound prefers RXRα/β over RXRγ. On the other hand, for NEt-3IB (**7c**) the ratio of EC_{50} (mean values) between RXRα:RXRβ:RXRγ was 0.58:23:3, indicating that this compound prefers RXRα over RXRβ. As a more than tenfold difference in EC_{50} values generally indicates the existence of selectivity,[20] it can be assumed that NEt-3IP (**7a**) is an RXRα/β-selective agonist (that is, RXRα/β dual agonist). NEt-3IB (**7c**) has less subtype selectivity than NEt-3IP (**7a**). NEt-3IP (**7a**) has comparable RXR potency to representative potent RXR pan agonists, LGD1069 (**3**) and PA024 (**5**), and NEt-3IB (**7c**) is

6a and **6b**, respectively (Table 1). These results suggested that application of alkoxy and isopropyl groups to the hydrophobic domain of RXR agonists enables the production of compounds with retinoid synergistic activities. Notably, the retinoid synergistic activity (SEC_{50} value) of NEt-3IP (**7a**) is 16 ± 1 nM, which is more effective than that of NEt-4IP (**7d**; 135 ± 15 nM). These results prompted us to conclude that the proper position for the introduction of alkoxy is at the 3-position. We therefore investigated the retinoid and retinoid synergistic activities of several derivatives possessing alkoxy groups at the 3-position. NEt-3IB (**7c**) possessing a branched alkoxy group, isobutoxy, was found to show potent retinoid synergistic activity without



Scheme 2. Reagents and conditions: a) H_2, Pd-C, MeOH, HCl. b) Ethyl 2-chloro-5-pyrimidinecarboxylic acid, K_2CO_3, DMF. c) EtI, NaH, DMF. d) NaOH, EtOH. e) HCl.

Table 1. Cell differentiation-inducing activity of compounds **8**–**10** by NBT reduction assay.[a]



Compd	OR	Retinoid activity		Retinoid synergist activity	
		EC_{50} [nM][b]	BA [%][c]	SEC_{50} [nM][d]	BA [%][c]
NEt-3IP (**7a**)	3′-O*i*Pr	>1000	n.d.[e]	16±1	83±1
NEt-3NP (**7b**)	3′-O*n*-Pr	>1000	n.d.	249±16	85±4
NEt-3IB (**7c**)	3′-O*i*Bu	>1000	n.d.	25±1	81±2
NEt-4IP (**7d**)	4′-O*i*Pr	>1000	n.d.	135±15	77±4
6a[d]	–	>1000	n.d.	309±33	67±5
6b[d]	–	>1000	n.d.	150±17	73±1

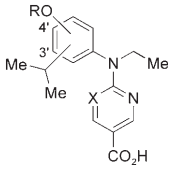
[a] All values were determined from full dose-response curves ranging from 10^{-9} to 10^{-5} M with HL-60 cells. Where errors are indicated, values represent the standard error of the mean value of at least two separate experiments. [b] EC_{50} or SEC_{50} was determined as the concentration of a test compound that required to elicit a response at half-maximal height on the dose-response curve. [c] Biological activity (%) is the maximal differentiation ratio that was induced by a test compound. [d] These data were quoted from reference [11]. [e] Not determinable.

more potent than those representative potent RXR pan agonists. PEt-3IP (**8a**) and PEt-3IB (**8c**) showed potent RXR agonistic activities, whereas their subtype selectivity was less than that of NEt series compounds. With increasing polarity of the acidic domain of the compounds, their RXR agonist activity increased but their subtype selectivity decreased. These results were nearly in accordance with the previous report,[11] suggesting that reduction of the hydrophobic interaction between the hydrophobic domain of the compounds and RXR-ligand binding domain is one strategy to produce subtype selectivity. The CLog*P* value of NEt-3IP (**7a**) is less than the values of NEt-3IB (**7c**), LGD1069 (**3**), and PA024 (**5**). These results indicate that NEt-3IP (**7a**) is a potent, less-lipophilic and subtype-selective RXR agonist.

Conclusions

To develop potent, less lipophilic and subtype-selective RXR agonists, we designed new RXR agonists possessing alkoxy and isopropyl groups as a lipophilic domain of the common structure of well-known RXR agonists. As a result, 6-[*N*-ethyl-*N*-(3-isopropoxy-4-isopropylphenyl)amino]nicotinic acid (NEt-3IP: **7a**) was discovered as the first RXRα/β-selective agonist. NEt-3IP (**7a**), being potent and having reduced lipophilicity and RXR subtype-selective activity, is expected to become a new medicinal product and to be a useful biological tool for clarifying each RXR subtype function. In the future, to evaluate the effectiveness of the compound, in vivo studies such as studies on oral absorption, disposition, toxicity, and anticancer activities are being planned.

Table 2. Co-transfection data for synthetic compounds and known RXR agonists **3** and **5** in COS-1 cells.[a]



Compd	OR	X	RXRα		RXRβ		RXRγ		Selectivity[e]		CLog*P*[f]
			EC_{50} [nM][b]	E_{max} [%][d]	EC_{50} [nM][b]	E_{max} [%][d]	EC_{50} [nM][b]	E_{max} [%][d]	β/α	γ/α	
NEt-3IP (**7a**)	3′-O*i*Pr	CH	32±0	136±11	36±8	115±9	376±13	96±6	1.1	11	5.61
NEt-3IB (**7c**)	3′-O*i*Bu	CH	0.58±0.02	114±4	23±10	140±13	3±1	103±6	39	5.1	6.23
NEt-4IP (**7d**)	4′-O*i*Pr	CH	410±40	112±11	1180±210	80±4	1430±30	81±15	2.8	3.4	5.61
PEt-3IP (**8a**)	3′-O*i*Pr	N	9±2	113±4	36±18	103±3	55±15	105±7	4.0	6.1	4.89
PEt-3IB (**8c**)	3′-O*i*Bu	N	4±2	106±2	5±0	144±14	4±0	105±13	1.2	1.0	5.50
6a[c]	–	–	195±25	115±16	2250±50	52±14	620±50	59±3	11	3.1	6.55
6b[c]	–	–	115±5	98±6	635±75	94±2	350±85	81±7	5.5	3.0	6.13
LGD1069 (**3**)	–	–	3±0	106±12	6±1	114±12	5±2	104±3	2.0	1.6	8.23
PA024 (**5**)[d]	–	–	3±1	–	24±0	–	8±1	—	8.0	2.6	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] These data were quoted from reference [11]. [d] Luciferase activity of PA024 (**5**) at 1 μM was defined as 100%. [e] Selectivity was calculated with each EC_{50} value. [f] CLog*P* values were calculated with ChemDraw Ultra7.0.

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 VarianVXR-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.

LGD1069 (3) and PA024 (5). These compounds were prepared according to references <[21] and [13], respectively.

2-Isopropyl-5-nitrophenol (10). Mixed acids (conc. HNO_3 : conc. H_2SO_4 = 2:5, 14 mL) were added to a solution of 2-isopropylaniline **9** (2.7 g, 20 mmol) in conc. H_2SO_4 (8.0 mL) through a dropping funnel with temperature maintained between −5 °C and 0 °C. The reaction mixture was alkalinized with 2N NaOH and extracted with EtOAc (3×200 mL). The organic layer was collected, washed with H_2O (200 mL) and brine

(150 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield 2.9 g of 2-isopropyl-5-nitroaniline as brown oil (81%). This compound gave a single spot on TLC, so it was used for the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.60 (1H, dd, J = 8.5, 2.5 Hz), 7.50 (1H, d, J = 2.5 Hz), 7.24 (1H, d, J = 8.5 Hz), 3.95 (2H, br s), 2.90 (1H, sep, J = 7.0 Hz), 1.29 ppm (6H, d, J = 7.0 Hz).

$NaNO_2$ (1.2 g, 18 mmol, dissolved in 3.0 mL of H_2O) was added to a suspension of 2-isopropyl-5-nitroaniline (2.9 g, 16 mmol) in H_2O (3.0 mL) and conc. H_2SO_4 (4.0 mL) with temperature maintained under 5 °C. The reaction status was checked by potassium iodide starch test paper. Then the reaction mixture was poured dropwise into a conc. H_2SO_4 (12 mL) and H_2O (9.0 mL) mixture at 120 °C. The reaction mixture was poured into H_2O (150 mL) and extracted with EtOAc (3×200 mL). The organic layer was collected, washed with H_2O (200 mL) and brine (150 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc : *n*-hexane = 1:5) to yield **10** as brown oil (2.2 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78 (1H, dd, J = 8.5, 2.5 Hz), 7.63 (1H, d, J = 2.5 Hz), 7.33 (1H, d, J = 8.5 Hz), 5.80 (1H, s), 3.31 (1H, sep, J = 7.0 Hz), 1.28 ppm (6H, d, J = 7.0 Hz).

2-Isopropoxy-1-isopropyl-4-nitrobenzene (13a). 2-Bromopropane (0.56 mL, 6.0 mmol), K_2CO_3 (552 mg, 3.6 mmol) and KI (catalytic amount) were added to a solution of **10** (668 mg, 3.7 mmol) in dry DMF (4.0 mL). The reaction mixture was stirred at 70 °C for 2 h. Then the reaction mixture was poured into H_2O (80 mL) and extracted with EtOAc (3×80 mL). The organic layer was collected, washed with H_2O (2×80 mL) and brine (80 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography to afford **13a** as light yellow oil (729 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.76 (1H, dd, J = 8.5, 2.0 Hz), 7.66 (1H, d, J = 2.0 Hz), 7.31 (1H, d, J = 8.5 Hz), 4.67 (1H, sep, J = 6.0 Hz), 3.36 (1H, sep, J = 7.0 Hz), 1.39 (6H, d, J = 6.0 Hz), 1.22 ppm (6H, d, J = 7.0 Hz).

1-Isopropyl-4-nitro-2-*n*-propoxybenzene (13b). 1-Iodopropane (595 mg, 3.5 mmol) and K_2CO_3 (414 mg, 3.0 mmol) were added to a solution of **10** (513 mg, 2.8 mmol) in dry DMF (4.0 mL). The reaction mixture was stirred at 80 °C overnight. Then the reaction mixture was poured into H_2O (50 mL) and extracted with EtOAc (3× 50 mL). The organic layer was collected, washed with H_2O (2× 50 mL) and brine (50 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **13b** as light yellow oil (426 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.80 (1H, dd, J = 8.5, 2.5 Hz), 7.65 (1H, d, J = 2.5 Hz), 7.31 (1H, d, J = 8.5 Hz), 4.02 (2H, t, J = 6.5 Hz), 3.39 (1H, sep, J = 7.0 Hz), 1.89 (2H, m), 1.24 (6H, d, J = 7.0 Hz), 1.09 ppm (3H, t, J = 7.5 Hz).

2-Isobutoxy-1-isopropyl-4-nitrobenzene (13c). Following the procedure to synthesize **13a**, **13c** was obtained in 71% yield as clear yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.80 (1H, dd, J = 8.5, 2.5 Hz), 7.65 (1H, d, J = 2.5 Hz), 7.32 (1H, d, J = 8.5 Hz), 3.82 (2H, d, J = 6.5 Hz), 3.40 (1H, sep, J = 7.0 Hz), 2.17 (1H, m), 1.25 (6H, d, J = 7.0 Hz), 1.08 ppm (6H, d, J = 6.5 Hz).

3-Isopropoxy-4-isopropylaniline acetate (14a AcOH). 10% activated Pd-C (catalytic amount) was added to a solution of **13a** (1.1 g, 4.9 mmol) in AcOH (4.0 mL). The reaction mixture was stirred under H_2 atmosphere at RT for 6 h. The reaction mixture was filtered through celite, and the celite cake was washed with EtOAc (100 mL). The solvent was evaporated under reduced pressure to give **14a** AcOH (q.y.). This compound gave a single spot on TLC, so it was used for the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.96 (1H, d, J = 8.0 Hz) 6.26 (1H, dd, J = 8.0, 2.0 Hz), 6.24 (1H, d, J = 2.0 Hz), 4.46 (1H, sep, J = 6.0 Hz), 3.19 (1H, sep, J = 7.0 Hz), 1.32 (6H, d, J = 6.0 Hz), 1.15 ppm (6H, d, J = 7.0 Hz).

4-Isopropyl-3-*n*-propoxyaniline acetate (14b AcOH). Following the procedure to synthesize **14a** AcOH, **14b** AcOH was obtained (q.y.). This compound gave a single spot on TLC, so it was used for the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.00 (1H, d, J = 8.0 Hz), 6.36 (1H, dd, J = 8.0, 2.0 Hz), 6.31 (1H, d, J = 2.0 Hz), 3.87 (2H, t, J = 6.5 Hz), 3.22 (1H, sep, J = 7.0 Hz), 1.81 (2H, m), 1.17 (6H, d, J = 7.0 Hz), 1.05 ppm (3H, t, J = 7.5 Hz).

3-Isobutoxy-4-isopropylaniline acetate (14c AcOH). Following the procedure to synthesize **14a** AcOH, **14c** AcOH was obtained (q.y.). This compound gave a single spot on TLC, so it was used for the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.97 (1H, d, J = 8.0 Hz), 6.27 (1H, dd, J = 8.0, 2.0 Hz), 6.23 (1H, d, J = 2.0 Hz), 3.67 (2H, d, J = 6.5 Hz), 3.22 (1H, sep, J = 7.0 Hz), 2.10 (1H, m), 1.18 (6H, d, J = 7.0 Hz), 1.04 ppm (6H, d, J = 7.0 Hz).

2-Isopropyl-4-nitrophenol (12). Conc. HNO_3 (2.5 mL, 20 mmol) was added to a solution of 2-isopropylphenol **11** (2.7 g, 20 mmol) in EtOAc (50 mL) in an ice bath. The reaction mixture was placed in an ultrasonic reactor, and $ZnCl_2$ (3.43 g, 25 mmol) was added in small portions over 2.5 h. The reaction mixture was poured into H_2O (100 mL) and extracted with EtOAc (3×100 mL). The organic layer was collected, washed with H_2O (2×100 mL) and brine (100 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield **12** (972 mg, 27%) as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.13 (1H, d, J = 3.0 Hz), 8.01 (1H, dd, J = 9.0, 3.0 Hz), 6.82 (1H, d, J = 9.0 Hz), 5.74 (1H, s), 3.25 (1H, sep, J = 7.0 Hz), 1.30 ppm (6H, d, J = 7.0 Hz).

1-Isopropoxy-2-isopropyl-4-nitrobenzene (13d). Following the procedure to synthesize **13a**, **13d** was obtained as brown oil (94%). This compound gave a single spot on TLC, so it was used for the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.10 (1H, d, J = 3.0 Hz), 8.07 (1H, dd, J = 8.5, 3.0 Hz), 6.86 (1H, d, J = 8.5 Hz), 4.70 (1H, sep, J = 6.0 Hz), 3.31 (1H, sep, J = 7.0 Hz), 1.40 (6H, d, J = 6.0 Hz), 1.24 ppm (6H, d, J = 7.0 Hz).

4-Isopropoxy-3-isopropylaniline (14d AcOH). Following the procedure to synthesize **14a** AcOH, **14d** AcOH was obtained in crude oil (q.y.). This compound gave a single spot on TLC, so it was used for the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.71 (1H, d, J = 8.5 Hz), 6.64 (1H, d, J = 3.0 Hz), 6.53 (1H, dd, J = 8.5, 3.0 Hz), 4.35 (1H, sep, J = 6.0 Hz), 3.28 (1H, sep, J = 7.0 Hz), 1.30 (6H, d, J = 6.0 Hz), 1.17 ppm (6H, d, J = 7.0 Hz).

4-[*N*-(3-Isopropoxy-4-isopropylphenyl)amino]nicotinic acid methyl ester (15a). 6-Chloronicotinic acid (788 mg, 5.0 mmol) was added to a solution of **14a** AcOH (1.24 g, 4.9 mmol) in AcOH (4.0 mL). The reaction mixture was stirred at 80 °C for 4 h. The reaction mixture was evaporated under reduced pressure. Conc. H_2SO_4 (catalytic amount) was added to a solution of the residue in dry MeOH (5.0 mL) under ice cooling. The reaction mixture was refluxed overnight. The reaction mixture was poured into sat. $NaHCO_3$ (100 mL) and extracted with EtOAc (2×50 mL). The organic layer was collected, washed with H_2O (50 mL) and brine (50 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **15a** (702 mg, 44%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.75 (1H, d, J = 2.5 Hz), 8.08 (1H, dd, J = 9.0, 2.5 Hz), 7.69 (1H, br s), 7.19 (1H, d, J = 8.0 Hz), 6.83 (1H, d, J = 9.0 Hz), 6.82 (1H, d, J = 2.0 Hz), 6.81 (1H, dd, J = 8.0, 2.0 Hz), 4.51 (1H, sep, J = 6.0 Hz), 3.90 (3H, s), 3.32 (1H, sep, J = 7.0 Hz), 1.35 (6H, d, J = 6.0 Hz), 1.21 ppm (6H, d, J = 7.0 Hz).

4-[*N*-(4-Isopropyl-3-*n*-propoxyphenyl)amino]nicotinic acid methyl ester (15b). Following the procedure to synthesize **15a**, **15b** was obtained as a purple solid (51% for 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.82 (1H, d, J=2.0 Hz), 8.03 (1H, dd, J=9.0, 2.0 Hz), 7.18 (1H, d, J=8.5 Hz), 6.89 (1H, s), 6.85 (1H, d, J=2.0 Hz), 6.84 (1H, dd, J=8.5, 2.0 Hz), 6.79 (1H, d, J=9.0 Hz), 3.91 (2H, t, J=6.5 Hz), 3.88 (3H, s), 3.31 (3H, sep, J=7.0 Hz), 1.84 (2H, m), 1.23 (6H, d, J=7.0 Hz), 1.07 ppm (3H, t, J=7.5 Hz).

4-[*N*-(3-Isobutoxy-4-isopropylphenyl)amino]nicotinic acid methyl ester (15c). Following the procedure to synthesize **15a**, **15c** was obtained as purple solid (62% for 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.82 (1H, d, J=2.5 Hz), 8.03 (1H, dd, J=9.0, 2.5 Hz), 7.18 (1H, d, J=8.5 Hz), 6.85 (1H, s), 6.84 (1H, dd, J=8.5, 2.0 Hz), 6.83 (1H, d, J=2.0 Hz), 6.79 (1H, d, J=9.0 Hz), 3.89 (3H, s), 3.71 (2H, d, J=6.5 Hz), 3.32 (1H, sep, J=7.0 Hz), 2.13 (1H, m), 1.23 (6H, d, J=7.0 Hz), 1.06 ppm (1H, d, J=6.5 Hz).

6-[*N*-(4-Isopropoxy-3-isopropylphenyl)amino]nicotinic acid methyl ester (15d). Following the procedure to synthesize **15a**, **15d** was obtained as white solid (57% for 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.75 (1H, d, J=2.5 Hz), 8.03 (1H, dd, J=9.0, 2.5 Hz), 7.40 (1H, br s), 7.09 (1H, d, J=3.0 Hz), 7.06 (1H, d, J=8.0, 3.0 Hz), 6.85 (1H, d, J=8.0 Hz), 6.67 (1H, d, J=9.0 Hz), 4.54 (1H, sep, J=6.0 Hz), 3.89 (3H, s), 3.32 (1H, sep, J=7.0 Hz), 1.36 (6H, d, J=6.0 Hz), 1.19 ppm (6H, d, J=7.0 Hz).

4-[*N*-Ethyl-*N*-(3-isopropoxy-4-isopropylphenyl)amino]nicotinic acid (7a). 15a (115 mg, 0.35 mmol) was added to a suspension of NaH (16 mg, 0.40 mmol, 60% dispersion in oil) in dry DMF (1.0 mL) at RT under argon. After stirring for 5 min, iodoethane (30 μL, 0.40 mmol) was added, and then it was stirred overnight. The reaction mixture was poured into H_2O (20 mL) and extracted with EtOAc (2×10 mL). The organic layer was collected, washed with H_2O (10 mL) and brine (10 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield a yellow oil. 2N NaOH (0.50 mL) was added to a solution of the residue in MeOH (2.0 mL), and it was stirred at 60 °C for 1 h. The reaction mixture was evaporated under reduced pressure to remove MeOH. The solution was poured into sat. NH_4Cl (20 mL) and extracted with EtOAc (3×10 mL). The organic layer was collected, washed with H_2O (2×10 mL) and brine (10 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. Recrystallization from MeOH afforded **7a** as colorless needles (48 mg, 40% for 2 steps). Mp: 212.0–214.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.91 (1H, d, J=2.0 Hz), 7.83 (1H, dd, J=9.0, 2.5 Hz), 7.26 (1H, d, J=8.0 Hz), 6.74 (1H, dd, J=8.0, 2.0 Hz), 6.65 (1H, d, J=2.0 Hz), 6.26 (1H, d, J=9.0 Hz), 4.49 (1H, sep, J=6.0 Hz), 4.06 (2H, q, J=7.0 Hz), 3.32 (1H, sep, J=7.0 Hz), 1.34 (6H, d, J=6.0 Hz), 1.25 (6H, d, J=7.0 Hz), 1.24 ppm (3H, t, J=7.0 Hz); IR (KBr): ν-=1698 cm^{-1}; FAB-MS *m/z*; 343 $[M+H]^+$; Anal. Calcd for $C_{20}H_{26}N_2O_3$: C, 70.15; H, 7.65; N, 8.18. Found: C, 70.18; H, 7.71; N, 8.46.

4-[*N*-Ethyl-*N*-(4-isopropyl-3-*n*-propoxyphenyl)amino]nicotinic acid (7b). Following the procedure to synthesize **7a**, **7b** was obtained as off-white cubics after being recrystallized from CH_2Cl_2/*n*-hexane (62% for 2 steps). Mp: 147.0–148.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.91 (1H, d, J=2.0 Hz), 7.86 (1H, dd, J=9.0, 2.0 Hz), 7.28 (1H, d, J=8.0 Hz), 6.76 (1H, dd, J=8.0, 2.0 Hz), 6.64 (1H, d, J=2.0 Hz), 6.29 (1H, d, J=9.0 Hz), 4.13 (2H, q, J=7.5 Hz), 3.88 (2H, t, J=6.5 Hz), 3.35 (1H, sep, J=7.0 Hz), 1.84 (2H, m), 1.27 (3H, t, J=7.5 Hz), 1.26 (6H, d, J=7.0 Hz), 1.07 ppm (3H, t, J=7.5 Hz); IR (KBr): $\tilde{\nu}$=2963, 1682 cm^{-1}; FAB-MS *m/z*: 343 $[M+H^+]$; Anal. Calcd for $C_{20}H_{26}N_2O_3$: C, 70.15; H, 7.65; N, 8.18. Found: C, 69.91; H, 7.61; N, 8.13.

4-[*N*-Ethyl-*N*-(3-isobutoxy-4-isopropylphenyl)amino]nicotinic acid (7c). Following the procedure to synthesize **7a**, **7c** was obtained as colorless needles after being recrystallized from CH_2Cl_2/*n*-hexane (41% for 2 steps). Mp: 191.5–193.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.91 (1H, d, J=2.0 Hz), 7.83 (1H, dd, J=9.0, 2.0 Hz), 7.26 (1H, d, J=8.0 Hz), 6.77 (1H, dd, J=8.0, 2.0 Hz), 6.64 (1H, d, J=2.0 Hz), 6.25 (1H, d, J=9.0 Hz), 4.04 (2H, q, J=7.0 Hz), 3.68 (2H, d, J=6.5 Hz), 3.36 (1H, sep, J=7.0 Hz), 2.12 (1H, m), 1.26 (6H, d, J=7.0 Hz), 1.25 (3H, t, J=7.0 Hz), 1.06 ppm (6H, d, J=6.5 Hz); IR (KBr): $\tilde{\nu}$=2960, 1684 cm^{-1}; FAB-MS *m/z*: 357 $[M+H^+]$; Anal. Calcd for $C_{21}H_{28}N_2O_3$: C, 70.76; H, 7.86; N, 7.92. Found: C, 70.92; H, 7.90; N, 7.89.

4-[*N*-Ethyl-*N*-(4-isopropoxy-3-isopropylphenyl)amino]nicotinic acid (7d). Following the procedure to synthesize **7a**, **7d** was obtained as off-white cubics after being recrystallized from *n*-hexane (54% for 2 steps). Mp: 212.0–214.0 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ=12.45 (1H, br s), 8.65 (1H, d, J=2.5 Hz), 7.77 (1H, dd, J=9.0, 2.0 Hz), 7.04 (3H, m), 6.14 (1H, d, J=9.0 Hz), 4.93 (2H, q, J=7.0 Hz), 4.64 (1H, sep, J=6.0 Hz), 3.23 (1H, sep, J=7.0 Hz), 1.31 (6H, d, J=6.0 Hz), 1.15 (6H, d, J=7.0 Hz), 1.14 ppm (3H, t, J=7.0 Hz); IR (KBr): $\tilde{\nu}$1664 cm^{-1}; FAB-MS *m/z*: 343 $[M+H^+]$; Anal. Calcd for $C_{20}H_{26}N_2O_3$: C, 70.15; H, 7.65; N, 8.18. Found: C, 70.02; H, 7.36; N, 8.23.

3-Isopropoxy-4-isopropylaniline hydrochloride (14a HCl). 10% activated Pd-C (catalytic amount) was added to a solution of **13a** (1880 mg, 8.4 mmol) in MeOH (15 mL). The reaction mixture was stirred under H_2 atmosphere at RT for 4 h. The reaction mixture was filtered through celite, and the celite cake was washed with MeOH. The solution was concentrated under reduced pressure. Conc. HCl (0.5 mL) and EtOAc (30 mL) were added to the concentrated solution. The precipitate was filtered to give colorless needles (1915 mg) containing **14a** HCl (99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=9.84 (2H, br s), 7.23 (1H, d, J=8.0 Hz), 6.89 (1H, s), 6.81 (1H, d, J=8.0 Hz), 4.53 (1H, sep, J=6.0 Hz), 3.19 (1H, sep, J=7.0 Hz), 1.30 (6H, d, J=6.0 Hz), 1.14 ppm (d, J=7.0 Hz).

3-Isobutoxy-4-isopropylaniline hydrochloride (14c HCl). Following the procedure to synthesize **14a** HCl, **14c** HCl was obtained as colorless needles (89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.98 (2H, br s), 7.23 (1H, d, J=8.0 Hz), 6.81 (1H, s), 6.80 (1H, d, J=8.0 Hz), 3.73 (2H, d, J=6.5 Hz), 3.22 (1H, sep, J=6.5 Hz), 2.08 (1H, m), 1.16 (6H, d, J=6.5 Hz), 1.02 ppm (6H, d, J=6.5 Hz).

2-[*N*-(4-Isopropoxy-3-isopropylphenyl)amino]pyrimidine-5-carboxylic acid ethyl ester (16a). K_2CO_3 (622 mg, 4.5 mmol) and DMF (5 drops) were added to a mixture of 2-chloropyrimidine-5-carboxylate (120 mg, 0.60 mmol) and **14a** HCl (148 mg, 0.60 mmol). The mixture was stirred at 120 °C for 12 h. The mixture was poured into 2N HCl (30 mL) and extracted with EtOAc (2×30 mL). The organic layer was collected, washed with H_2O (2×30 mL) and brine (20 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane=1:6) to yield **16a** (152 mg, 69%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.95 (2H, s), 7.39 (1H, br s), 7.34 (1H, d, J=2.0 Hz), 7.16 (1H, d, J=8.0 Hz), 6.99 (1H, dd, J=8.0, 2.0 Hz), 4.56 (1H, sep, J=6.0 Hz), 4.37 (2H, q, J=7.0 Hz), 3.28 (1H, sep, J=7.0 Hz), 1.39 (3H, t, J=7.0 Hz), 1.37 (6H, d, J=6.0 Hz), 1.20 ppm (6H, d, J=7.0 Hz).

2-[*N*-(4-Isobutoxy-3-isopropylphenyl)amino]pyrimidine-5-carboxylic acid ethyl ester (16c). Following the procedure to synthesize **16a**, **16c** was obtained as a colorless solid (33%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.95 (2H, s), 7.46 (1H, s), 7.26 (1H, d, J=2.5 Hz), 7.17 (1H, d, J=8.5 Hz), 7.03 (1H, dd, J=8.5, 2.5 Hz), 4.38 (2H, q, J=7.0 Hz), 3.76 (2H, d, J=6.5 Hz), 3.31 (1H, m), 2.44 (1H, sep), 1.39 (3H, t, J=7.0 Hz), 1.22 (6H, d, J=7.0 Hz), 1.07 ppm (6H, d, J=7.0 Hz).

2-[*N*-Ethyl-*N*-(4-isopropoxy-3-isopropylphenyl)amino]pyrimidine-5-carboxylic acid (8a). A solution of **16a** (40 mg, 0.12 mmol) was added to a suspension of NaH (8 mg, 0.20 mmol) in DMF (2.0 mL)

under Ar atmosphere. The solution was stirred at RT for 10 min, and then EtI (10 μL, 0.12 mmol) was added, and stirring was continued for an additional 10 min. The solution was poured into H_2O (20 mL) and extracted with EtOAc (2×15 mL). The organic layer was collected, washed with H_2O (2×20 mL) and brine (10 mL), and dried over $MgSO_4$. The solvent was evaporated to yield 38 mg of colorless solid. 2 N NaOH (2.0 mL) was added to a solution of the residue (35 mg, 0.10 mmol) in EtOH (2.0 mL). The mixture was stirred at 60 °C for 10 min. The solution was neutralized with 2 N HCl and extracted with EtOAc (2×15 mL). The organic layer was collected, washed with H_2O (2×20 mL) and brine (10 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield **8a** (32 mg, 88% for 2 steps) as a colorless solid. Mp: 197.5–199.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.74 (2H, s), 7.21 (1H, d, J = 8.0 Hz), 6.83 (1H, d, J = 2.0 Hz), 6.77 (1H, dd, J = 8.0, 2.0 Hz), 4.56 (1H, sep, J = 6.0 Hz), 4.00 (2H, q, J = 7.0 Hz), 2.11 (1H, sep, J = 7.0 Hz), 1.26 (6H, d, J = 6.0 Hz), 1.19 (6H, d, J = 7.0 Hz), 1.16 ppm (3H, t, J = 7.0 Hz); IR (KBr): $\tilde{\nu}$1674 cm^{-1}; FAB-MS m/z: 344 [M+H$^+$]; Anal. Calcd for $C_{19}H_{25}N_3O_3$: C, 66.45; H, 7.34; N, 12.24. Found: C, 66.38; H, 7.29; N, 12.43.

2-[*N*-Ethyl-*N*-(4-isobutoxy-3-isopropylphenyl)amino]pyrimidine-5-carboxylic acid (8c). Following the procedure to synthesize **8a**, **8c** was obtained as colorless needles (69% for 2 steps). Mp: 180.5–182.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.89 (2H, s), 7.26 (1H, d, J = 8.0 Hz), 6.79 (1H, dd, J = 8.0, 2.0 Hz), 6.66 (1H, d, J = 2.0 Hz), 4.06 (2H, q, J = 7.0 Hz), 3.69 (2H, d, J = 6.5 Hz), 3.34 (1H, m), 2.11 (1H, sep), 1.28 (3H, t, J = 7.0 Hz), 1.25 (6H, d, J = 7.0 Hz), 1.05 ppm (6H, d, J = 6.5 Hz); IR (KBr): ν- = 1673 cm^{-1}; Anal. Calcd for $C_{20}H_{27}N_3O_3$: C, 67.20; H, 7.61; N, 11.76. Found: C, 67.01; H, 7.25; N, 11.60.

Calculation of CLog*P* Values. Log*P* values for compounds were calculated with ChemDraw Ultra 7.0.

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.[10,16,17] Test compounds were dissolved in DMSO at 20 mM for stock solutions. A test compound solution in DMSO was added to a suspension of cells at a concentration of 8×10^4 cells mL^{-1}. Final DMSO concentration was kept below 0.1%. For vehicle and positive controls, the same volume of DMSO and Am80[8] 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. The 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.[13,18,19] 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 DNA was 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 4×10^4 cells mL^{-1} in 96-well white plates. For vehicle and positive controls, 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, some of the medium was 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.

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[1] L. Altucci, M. D. Leibowitz, K. M. Ogilvie, A. R. D. Lera, H. Gronemeyer, *Nat. Rev. Drug. Discov.* **2007**, *6*, 793–810.
[2] A. R. de Lera, W. Bourguet, L. Altucci, H. Gronemeyer, *Nat. Rev. Drug. Discov.* **2007**, *6*, 811–820.
[3] C. J. Grubbs, D. L. Hill, K. I. Bland, S. W. Beenken, T.-H. Lind, I. Eto, V. R. Atigadda, K. K. Vines, W. J. Brouillette, D. D. Muccio, *Cancer Lett.* **2003**, *201*, 17–24.
[4] W.-C. Yen, M. R. Corpuz, R. Y. Prudente, T. A. Cooke, R. P. Bissonnette, A. Negro-Vilar, W. W. Lamph, *Clin. Cancer Res.* **2004**, *10*, 8656–8664.
[5] V. Giguére, *Endocr. Rev.* **1999**, *20*, 689–725.
[6] D. J. Mangelsdorf, C. Thummel, M. Beato, P. Herrlich, G. Schütz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, R. M. Evans, *Cell* **1995**, *83*, 835–839.
[7] D. J. Mangelsdorf, R. M. Evans, *Cell* **1995**, *83*, 841–850.
[8] A. Szanto, V. Narkar, Q. Shen, I. P. Uray, P. J. A. Davies, L. Nagy, *Cell Death Differ.* **2004**, *11*, S126–S143.
[9] P. F. Egea, A. Mitschler, D. Moras, *Mol. Endocrinol.* **2002**, *16*, 987–997.
[10] H. Kagechika, K. Shudo, *J. Med. Chem.* **2005**, *48*, 5875–5883.
[11] K. Takamatsu, A. Takano, N. Yakushiji, K. Morishita, N. Matsuura, M. Makishima, H. I. Ali, E. Akaho, A. Tai, K. Sasaki, H. Kakuta, *ChemMedChem*, in press.
[12] A. Kamal, B. A. Kumar, M. Arifuddin, M. Patrick, *Ultrasonics Sonochemistry* **2004**, *11*, 455–457.
[13] K. Ohta, E. Kawachi, N. Inoue, H. Fukasawa, Y. Hashimoto, A. Itai, H. Kagechika, *Chem. Pharm. Bull.* **2000**, *48*, 1504–1513.

[14] A. Takamizawa, K. Tokuyama, H. Satoh, *Yakugaku Zasshi* **1959**, *79*, 664–669.
[15] L. Nagy, V. A. Thomázy, G. L. Shipley, L. Fésüs, W. Lamph, R. A. Heyman, R. A. S. Chandraratna, P. J. A. Davies, *Mol. Cell. Biol.* **1995**, *15*, 3540–3551.
[16] S. J. Collins, F. W. Ruscetti, R. E. Gallagher, R. C. Gallo, *J. Exp. Med.* **1979**, *149*, 969–974.
[17] T. Takuma, K. Takeda, K. Konno, *Biochem. Biophys. Res. Commun.* **1987**, *145*, 514–521.
[18] K. Umesono, K. K. Murakami, C. C. Thompson, R. M. Evans, *Cell* **1991**, *65*, 1255–1266.
[19] S. R. Kain, *Methods Mol. Biol.* **1997**, *63*, 49–60.
[20] L. Ye, Y.-L. Li, K. Mellström, C. Mellin, L. G. Bladh, K. Koehler, N. Garg, A. M. G. Collazo, C. Litten, B. Husman, K. Persson, J. Ljunggren, G. Grover, P. G. Sleph, R. George, J. Malm, *J. Med. Chem.* **2003**, *46*, 1580–1588.
[21] M. F. Boehm, L. Zhang, B. A. Badea, S. K. White, D. E. Mais, E. Berger, C. M. Suto, M. E. Goldman, R. A. Heyman, *J. Med. Chem.* **1994**, *37*, 2930–2941.

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