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Design, Synthesis, and Structure−Activity Relationship Studies of Novel 2,4,6-Trisubstituted-5-pyrimidinecarboxylic Acids as Peroxisome Proliferator-Activated Receptor γ (PPARγ) Partial Agonists with Comparable Antidiabetic Efficacy to Rosiglitazone

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A series of novel 2,4,6-trisubstitutedpyrimidine-5-carboxylic acid derivatives were designed and synthesized with the intent of producing a peroxisome proliferator-activated receptor γ (PPARγ) partial agonist for antidiabetic agents. A pharmacophore-driven approach of in-house screening identified compound **7**, which led to the identification of compound **9** featuring a 2,4,6-trisubstituted pyrimidine-5-carboxylic acid core. Structure−activity relationship studies of **9** resulted in identifying 4,6-bisbenzylthio-2-methylthiopyrimidine-5-carboxylic acid (**50**) as the most attractive of all the screened compounds. The X-ray cocrystal structure of **50** bound on PPARγ revealed that the key hydrogen bond interactions, which are not related to the activation function 2 (AF-2) site, are different from those of the full agonist. Compound **50** showed typical PPARγ partial agonist properties in the PPARγ-GAL4 functional assay and weaker differentiation of adipocytes in 3T3-L1 cells than observed with rosiglitazone. Furthermore, **50** displayed comparable antidiabetic efficacy with rosiglitazone in db/db mice, although its potency is 10-fold weaker than that of rosiglitazone.

Introduction

The peroxisome proliferator-activated receptors (PPARs)[a] are ligand-activated transcription factors belonging to the nuclear receptor superfamily.[1] So far, there are three PPAR subtypes encoded by distinct genes: PPARα (NR1C1), PPARδ (NR1C2), and PPARγ (NR1C3).[2] These receptors are important regulators in multiple physiological pathways such as glucose homeostasis, fatty acid metabolism, inflammation, and cellular differentiation.[3,4]

PPARγ is also the target protein for the currently marketed thiazolidinedione (TZD) class of antidiabetic agents, rosiglitazone and pioglitazone.[5,6] Although this type of PPARγ full agonist modestly ameliorates hyperglycemia, undesirable side effects including peripheral edema and weight gain are also associated with these drugs.[7,8] Therefore, a recent drug-discovery program has focused on identifying PPARγ partial agonists that promise to surpass the potential of currently available PPARγ full agonists if their desirable efficacy profile can be maintained, while their negative side effects are minimized.[9−13] On the basis of this concept, several PPARγ partial agonists have been developed.[14−19]

Structurally, the majority of the known PPAR partial agonists can be classified into three types, types A−C in Figure 1. Type A, which includes **1** (balaglitazone),[14] **2** (FK-614),[15] and **3** (MK-0533),[16] has similar structural features as PPARγ full agonists such as rosiglitazone, which is made up of an acidic head part and a lipophilic tail part. Compound **1** is currently included in an ongoing phase III clinical trial. Type B, which includes **4** (metaglitazen)[17] and **5** (nTZDpa),[9] has two lipophilic parts on either side of an acidic center. Compound **4**, the most advanced drug candidate of this type, is currently included in a phase III clinical trial. The acidic parts of the Type A and Type B partial agonists are known to be important for key hydrogen bond interactions with PPARγ.[20] In contrast, Type C, which includes **6** (L-764406),[21] usually has no acidic part to form hydrogen bonds with PPARγ. This type of partial agonist has a reactive leaving group on an electron-deficient ring; this leaving group is important to the formation of covalent interactions with PPARγ. Compound **6** is known to bind directly to Cys313 in helix 3 of the LBD of human PPARγ through the loss of a chlorine atom. To the best of our knowledge, there has been no report of a clinical trial involving Type C partial agonists.

Our research on PPARγ partial agonists has been initiated from our screening identification of compound **7**, which possesses a new pyrimidine-5-carboxamide skeleton, as shown in Scheme 1. On the basis of the chemical structure, **7** can probably be categorized as a Type C partial agonist and can interact with PPARγ in a covalent fashion through the loss of chlorine. As expected, compound **8**, a related analogue compound lacking the chlorine, was inactive in this assay. Covalent binding may cause toxicological risks over the long-term;[22]

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[a]Abbreviations: PPAR, peroxisome proliferator-activated receptor; AF-2, activation function 2, TZD, thiazolidinedione; LDA, lithium diisopropylamide; LBD, ligand-binding domain; aP2, adipose fatty acid binding protein; PCR, polymerase chain reaction; IBMX, 3-isobutyl-1-methylxanthine; DEX, dexamethazone, MIX, methylisobutyl xanthine; SEM, standard error of the mean; DMF, dimethylformamide; THF, tetrahydrofuran; *m*CPBA, *meta*-chloroperoxybenzoic acid

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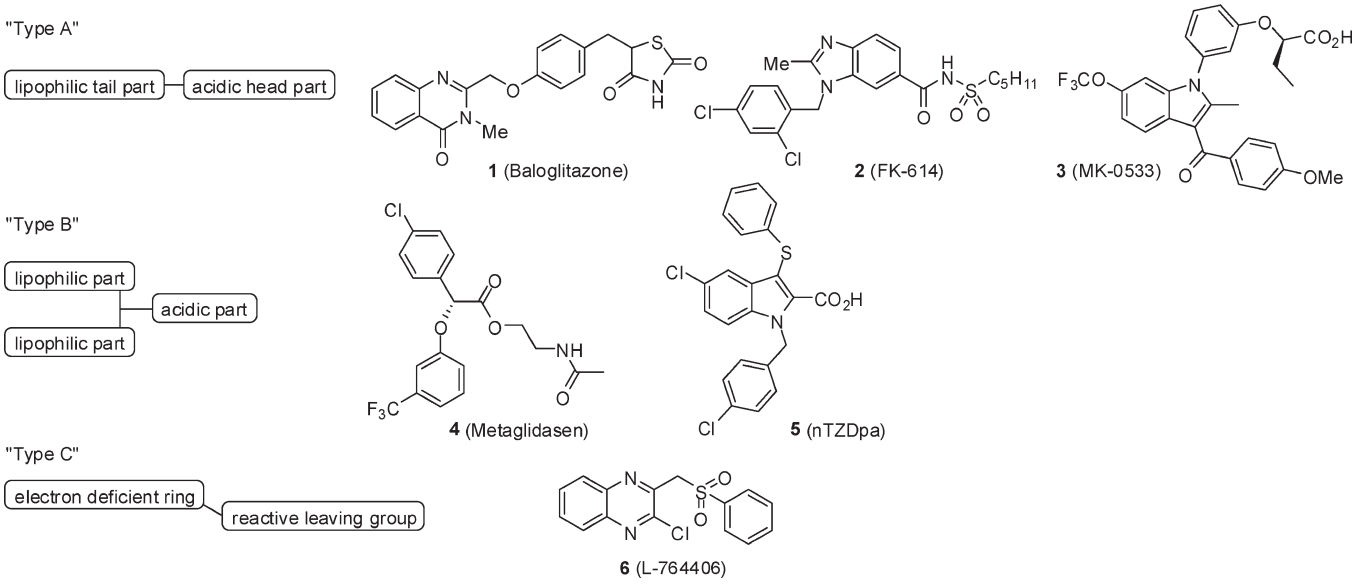
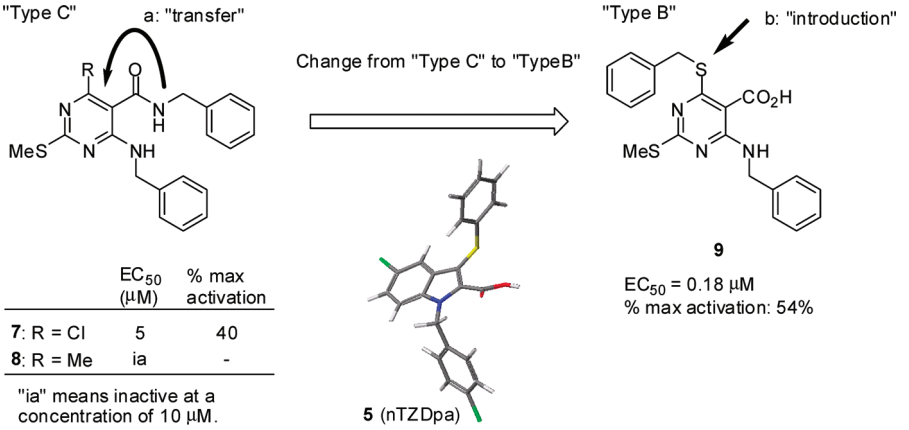


Figure 1. Structural classification of known PPARγ partial agonists.

Scheme 1



however, the novel structural features of compound **7** led us to embark on further investigation. With the intent of finding a noncovalent binder, we next focused our attention on the similarity of the chemical structure between **7** and **5**, which is categorized as Type B. We synthesized **9** by transference of the benzyl amide group of **7** to the 4-position on the pyrimidine ring and the introduction of a sulfur atom from one of the Type B compounds, **5**, as in Scheme 1. Obviously, **9** belongs to the Type B category, which has two lipophilic parts on either side of an acidic center, with no reactive leaving group. Moreover, **9** showed a 25-fold higher potency in the transactivation assay than **7**.

We wish to report herein the details of structure−activity relationship (SAR) efforts toward further optimization of a series of novel 2,4,6-trisubstituted-5-pyrimidinecarboxylic acid derivatives. We report the X-ray cocrystal structure of selected compounds bound to the PPARγ to reveal important molecular insight into the ligand-binding site interactions and encouraging in vivo efficacy results in db/db mice.

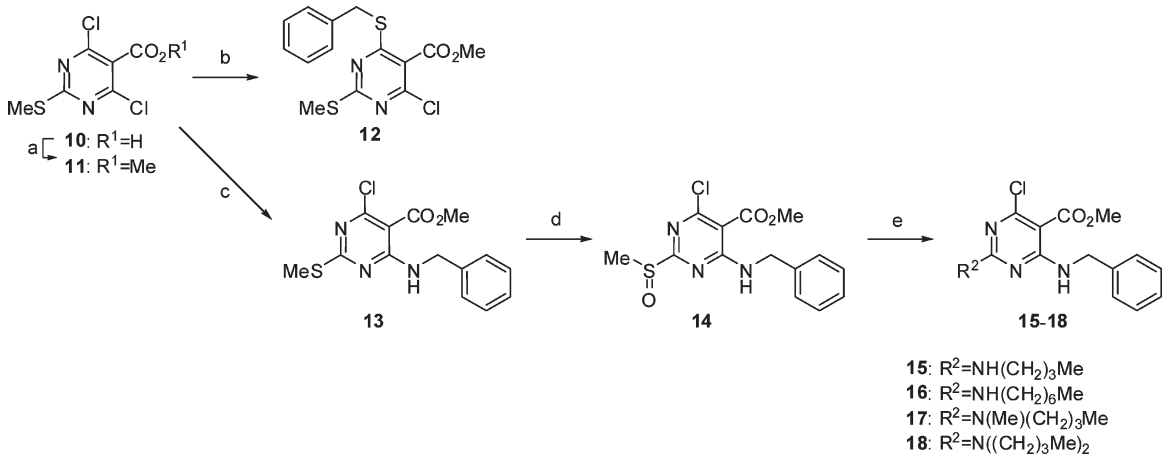
Chemistry

Chloropyrimidine analogues, common key intermediates, were synthesized as outlined in Scheme 2. The coupling of dichloride **11** with phenyl methanethiol under basic conditions afforded the benzylthio ether **12**. Similar conditions as those used in the preparation of **12** were used for the synthesis of benzylamine **13**. Further transformation of **13** took place in order to introduce various kinds of amino groups at C-2. Oxidation of the sulfur atom of **13** using *m*CPBA afforded methyl sulfone **14**; a subsequent displacement reaction of a sulfinyl group of **14** with various amine nucleophiles in toluene provided 2-aminopyrimidines **15**−**18**, which have a chlorine atom at the 4-position for further transformation.[23]

Some of the 2-alkylthioanalogues were prepared using the method described in Scheme 3. Alkylation[24] of barbituric acid **19** with butyl bromide or octyl bromide using sodium hydroxide afforded 2-alkylthiopyrimidines **20** and **21**, respectively. The introduction of a carboxylic acid moiety into **20** and **21** using CO_2 and LDA as well as subsequent methylation with iodomethane gave esters, which were coupled with phenyl methanethiol to provide thioethers **26** and **27**, respectively.

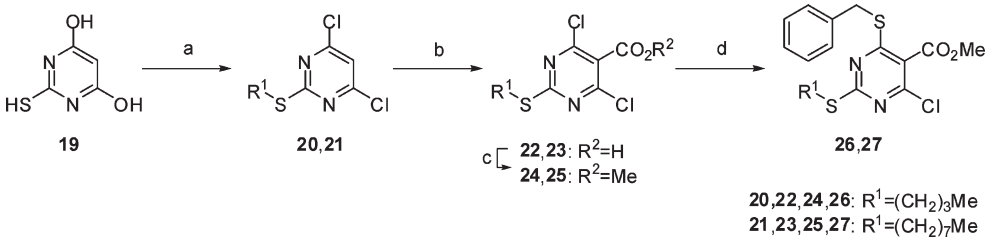
The trisubstituted pyrimidine **31** was prepared as shown in Scheme 4. Bell's condition[25] was used for the preparation of carboxylic acid **29**, which was transformed to thioether **31** by the methylation of **29** and a subsequent coupling reaction of **30** with phenyl methanethiol.

Scheme 2[a]



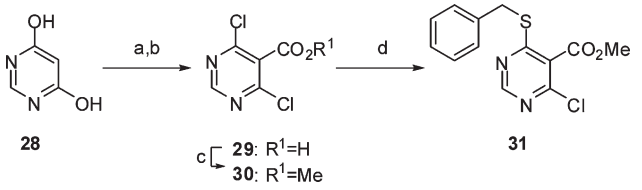
[a] Reagents: (a) MeI, K_2CO_3, DMF, rt, 1 h; (b) $PhCH_2SH$, Et_3N, THF, rt, 2 h; (c) $PhCH_2NH_2$, Et_3N, THF, rt, 2 h; (d) *m*CPBA, THF, 0 °C, 5 h; (e) R^2H, toluene, 0 °C, 5 h.

Scheme 3[a]



[a] Reagents: (a) (i) butyl bromide or octyl bromide, 3 N NaOH, EtOH, reflux, 2 h, (ii) $POCl_3$, *N*,*N*-dimethylaniline, reflux, 1 h; (b) (i) LDA, THF, −78 °C, 5 h, (ii) CO_2, 10 min; (c) MeI, K_2CO_3, DMF, rt, 2 h; (d) $PhCH_2SH$, K_2CO_3, DMF, rt, 1 h.

Scheme 4[a]



[a] Reagents: (a) $POCl_3$, DMF, 100 °C, 5 h; (b) $NaClO_2$, 2-methyl-2-butene, *t*-BuOH, 0 °C, 4 h; (c) MeI, K_2CO_3, DMF, rt, 2 h; (d) $PhCH_2SH$, K_2CO_3, DMF, rt, 1 h.

Having the chlorides **10**−**18**, **26**, **27**, **31** in hand, the target pyrimidine-5-carboxylic acids **9**, **32**−**50** were synthesized as shown in Schemes 5−7. The nucleophilic displacement reaction of chlorides **12**, **26**, **27**, **31** with various kinds of amines, and then subsequent hydrolysis with potassium hydroxide gave **9**, **32**−**43**, respectively (Scheme 5). Similarly, chlorides **13**, **15** underwent coupling with benzyl alcohol, phenyl methanethiol and subsequent hydrolysis with potassium hydroxide afforded the corresponding carboxylic acids **44**, **45** (Scheme 6). A one-pot procedure was used for the synthesis of the target compounds **46**−**48** by heating the reaction mixture of phenyl methanethiol and chloride **16**−**18** in DMF at 80 °C for 1 h.[26] Biaryl analogue **49** was prepared by using a Suzuki coupling reaction[27] of chloride **13** with 2-thienoboronic acid, and then subsequent hydrolysis with potassium hydroxide (Scheme 6). Scheme 7 shows the synthesis of symmetrical compound **50**. Bisbenzylthio ether **50** was prepared from the direct coupling between carboxylic acid **10** and 2 equiv of phenyl methanethiol.

Results and Discussion

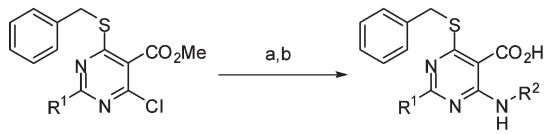
The PPARγ transactivation activation activities of the synthesized compounds were determined, and the results are summarized in Tables 1 and 2, together with the results for rosiglitazone. The PPARγ transactivation value was assessed by potency and efficacy using PPAR-GAL4 chimeric receptors in transiently transfected CHO-K1 cells. Efficacy values were calculated as a percentage of the maximal reporter activity value of rosiglitazone.

The results shown in Table 1 reveal the effects of the length and bulkiness of the alkyl chain of the R^1 group. The *des*-(methylthio) analogue **43** was found to be inactive on PPARγ, while the parent methylthio analogue **9** resulted in a significant activation of the receptor (EC_{50} = 0.18 μM). Further elongation of the alkyl chain of the R^1 group, the propyl group as in **41**, and the octyl group as in **42**, led to an increase in efficacy: 54% (**9**, methyl), 76% (**41**, butyl), and 89% (**42**, octyl), respectively, without a significant change in EC_{50}, which ranged from 0.18−0.95 μM. A similar tendency in efficacy was observed for the alkylamino derivatives **45** (65%, butyl) and **46** (85%, heptyl). These results suggest that the length of the alkyl chain is important for adjusting the efficacy of PPARγ activation. We anticipate that these compounds, with a variety of efficacies, may bind to different sites of PPARγ, on the basis of the previously reported binding mode of indole analogues with different efficacies.[20,28]

The bulky dibutylamino analogue **48** showed drastically reduced potency (EC_{50} > 10 μM) over monobutylamino analogue **45**.

The effects of the substituents in positions 4 and 6 of the pyrimidine nucleus were examined, as shown in Table 2.

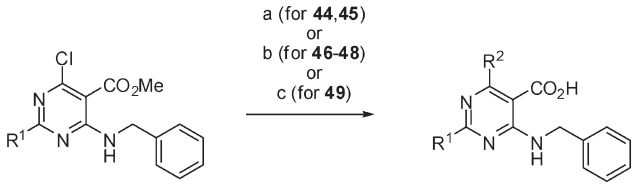
Scheme 5[a]



9: R^1=SMe, R^2=CH_2Ph
32: R^1=SMe, R^2=Ph
33: R^1=SMe, R^2=CH_2CH_2Ph
34: R^1=SMe, R^2=CH_2-2-pyridyl
35: R^1=SMe, R^2=CH_2-3-pyridyl
36: R^1=SMe, R^2=CH_2-4-pyridyl
37: R^1=SMe, R^2=Me
38: R^1=SMe, R^2=$(CH_2)_2Me$
39: R^1=SMe, R^2=cyclopropyl
40: R^1=SMe, R^2=CH_2-cyclohexyl
41: R^1=$S(CH_2)_3Me$, R^2=CH_2Ph
42: R^1=$S(CH_2)_7Me$, R^2=CH_2Ph
43: R^1=H, R^2=CH_2Ph

[a] Reagents: (a) R^2NH_2, K_2CO_3, DMF, rt, 2 h; (b) KOH, THF-MeOH, reflux, 4 h.

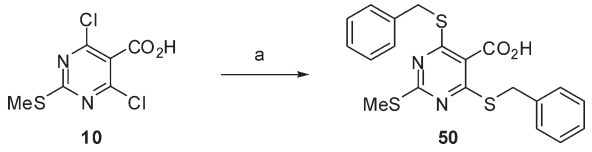
Scheme 6[a]



44: R^1=SMe, R^2=OCH_2Ph
45: R^1=$NH(CH_2)_3Me$, R^2=SCH_2Ph
46: R^1=$NH(CH_2)_6Me$, R^2=SCH_2Ph
47: R^1=$N(Me)(CH_2)_3Me$, R^2=SCH_2Ph
48: R^1=$N((CH_2)_3Me)_2$, R^2=SCH_2Ph
49: R^1=SMe, R^2=2-thieno

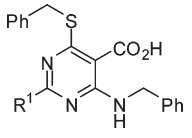
[a] Reagents: (a) (i) R^2H, K_2CO_3, DMF, rt, 1 h, (ii) KOH, THF-MeOH, reflux, 4 h; (b) $PhCH_2SH$, K_2CO_3, DMF, 80 °C, 3 h. (c) (i) $R^2B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, toluene, dioxane, H_2O, reflux, 2 h, (ii) KOH, THF-MeOH, reflux, 4 h.

Scheme 7[a]



[a] Reagents: (a) $PhCH_2SH$ or $PhCH_2OH$, K_2CO_3, DMF, rt, 1 h.

Table 1. Effect of C-2 Substituents

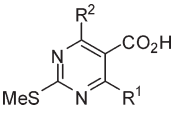


compd	R^1	EC_{50} (μM)[a]	efficacy (%)[b]
43	H	> 10	33
9	SMe	0.18	54
41	$S(CH_2)_3Me$	0.55	76
42	$S(CH_2)_7Me$	0.95	89
45	$NH(CH_2)_3Me$	0.47	65
46	$NH(CH_2)_6Me$	0.25	85
47	$N(Me)(CH_2)_3Me$	0.42	57
48	$N((CH_2)_3Me)_2$	> 10	71

[a] EC_{50} values were the molar concentration of the test compounds that cause 50% of the maximal reporter activity. [b] Efficacy values were calculated as a percentage of rosiglitazone.

We first examined the effects of the phenyl ring on the benzylamino moiety. The length of the linker between the terminal phenyl ring and the nitrogen atom attached to the core pyrimidine ring appears to play an important role in the potency. The anilino analogue **32** and the phenethylamino analogue **33** showed 9- and 4-fold less potency than the parent benzyl analogue **9**, respectively. The 2-pyridyl analogue **34** retained the same level of potency compared to **9**. Shifting the nitrogen on the pyridine ring to the 3- or 4-position resulted in a decrease in potency (4-fold, **35**; 15-fold, **36**).

Table 2. Effects of C-4 and 6 Substituents



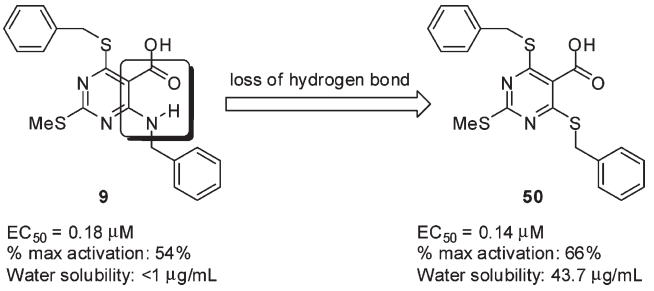
compd	R^1	R^2	EC_{50} (μM)[a]	efficacy (%)[b]
32	NHPh	SCH_2Ph	1.6	58
9	$NHCH_2Ph$	SCH_2Ph	0.18	54
33	$NHCH_2CH_2Ph$	SCH_2Ph	0.67	50
34	$NHCH_2$-2-pyridyl	SCH_2Ph	0.25	35
35	$NHCH_2$-3-pyridyl	SCH_2Ph	0.74	55
36	$NHCH_2$-4-pyridyl	SCH_2Ph	2.7	45
37	NHMe	SCH_2Ph	> 10	37
38	$NH(CH_2)_2Me$	SCH_2Ph	> 10	35
39	NH-cyclopropyl	SCH_2Ph	1.8	25
40	$NHCH_2$-cyclohexyl	SCH_2Ph	0.30	65
44	$NHCH_2Ph$	OCH_2Ph	2.3	50
49	$NHCH_2Ph$	2-thienyl	> 10	ia[c]
50	SCH_2Ph	SCH_2Ph	0.14	66
Rosiglitazone			0.10	100

[a] EC_{50} values were the molar concentration of the test compounds that cause 50% of the maximal reporter activity. [b] Efficacy values were calculated as a percentage of rosiglitazone. [c] "ia" means inactive at a concentration of 10 mM.

The introduction of various kinds of substituents onto the terminal phenyl ring resulted in comparable potency (see **S1**−**S10** in Supporting Information). For example, the 4-methoxy analogue, 4-methyl analogue, 4-chloro analogue, 4-phenyl analogue, 4-*tert*-butyl analogue, and 4-dimethylamino analogue showed similar potencies compared to **9** at the range of EC_{50} = 0.27−0.50, indicating that the presence of a substituent on the phenyl ring is not crucial to the interaction with PPARγ. Removal of the terminal phenyl ring negatively affected potency. Most of the alkyl analogues without a terminal aromatic ring, for example, **37** and **38** as well as cyclopropyl analogue **39**, did not respond to PPARγ. However, the cyclohexylmethyl analogue **40** maintained its potency at almost the same level as the parent **9** (EC_{50} = 0.30 μM).

Next, the effects of the benzylthio moiety (R^2) were examined. Conversion of the sulfur on the benzylthio moiety to oxygen negatively affected the potency (**9** vs **44**). Thienyl analogue **49**, a conformationally restricted analogue, showed a dramatic loss of activity.

Scheme 8



On the basis of the results of the SAR examination shown in Tables 1 and 2, parent compound **9** appears to have the greatest potency in this series of compounds. However, **9** showed extremely poor water solubility (less than 1 μg/mL), which could obstruct the absorption process during oral administration. We hypothesized that hydrogen bonding could occur between a carboxylic acid group and benzylamine NH of **9**, which could lead to low water solubility in Scheme 8. To test this hypothesis, we synthesized the bisbenzylthio analogue **50**. As a result, not only did **50** show good water solubility (43.7 μg/mL), it showed PPARγ agonist activity similar to that of **9**. We therefore selected **50** as the most attractive compound for further biological examinations.

To clarify the hydrogen bond network between **50** and PPARγ, an X-ray cocrystal structure of **50** bound to an active PPARγ site was obtained by X-ray crystallography to a resolution of 3.0 Å, as shown in Figure 2. Analysis of the binding mode reveals that the oxygen atom of the carboxylic acid part of **50** forms a hydrogen bond with the carboxylic acid side chain of Glu343; the distance between the two oxygen atoms is 2.95 Å. The oxygen atom of **50** also interacts with Arg288 through a hydrogen bond; the distance between the oxygen atom and the nitrogen atom of the guanidinium group of Arg288 is 2.76 Å. Thus, the acidic part of **50** does not directly interact with three key amino acid residues, H323, H449, and Y473, located on the C-terminal activation function 2 (AF-2) helix and interact with acidic head groups of synthetic PPARγ full agonists.[29]

The species difference of **50** in PPARγ transactivation was examined using dose response analysis in the human and mice PPARγ-GAL4 functional assay. As shown in Table 3, **50** showed a partial agonist profile to both human and mice PPARγ receptors with a relative efficacy of 50−60% of

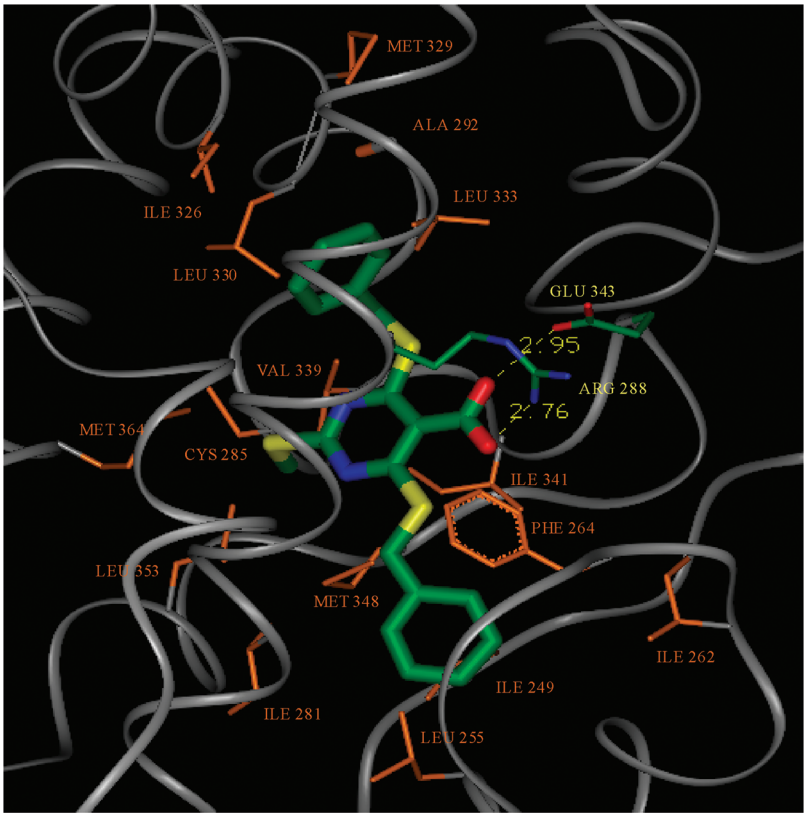


Figure 2. Hydrogen bond network of compound **50** in complex with PPARγ.

rosiglitazone. Compound **50** also showed an antagonistic profile with IC_{50} = 1.79 μM when treated in the presence of 1 μM rosiglitazone.[30] Similar results were observed when mice and rat PPARγ were used. These data indicate that **50** displayed typical PPARγ partial agonist properties without a species difference.

Partial agonists exhibit reduced ability to recruit coactivators, diminished adipogenic capacity, and attenuated gene signatures in cultured adipocytes.[31,32] To characterize the profile of **50**, we examined the ability to promote adipogenesis in 3T3L1 cells.[33] We first examined the levels of adipose fatty acid binding protein (aP2) mRNA expression in differentiating 3T3-L1 adipocytes (blast and mature), as shown in Figure 3.[34,35] Rosiglitazone, a full agonist, increased aP2 mRNA expression in a dose-dependent manner in both blast and mature 3T3-L1 cell lines. In contrast, **50** did not affect the expression of mRNA in blast cells. A partial increasing effect was observed in mature cells over rosiglitazone. Next, we also examined the effects of **50** on adipocyte differentiation in murine fibroblast 3T3-L1 cells, as shown in Figure 4. Rosiglitazone promoted lipid accumulation according to the results of oil red O staining, while the effects of **50** were found to be very weak, suggesting that **50** does not induce weight and adipose tissue gain.

Before conducting in vivo experiments in diabetic mice, the pharmacokinetic profile of **50** in mice was evaluated and the results are shown in Table 4. Compound **50** showed low total clearance (CL, 1.88 mL/min/kg), moderate oral bioavailability (35.8%), but a short half-life ($t_{1/2}$ = 0.76 h), suggesting that **50** is an orally bioavailable compound.

The in vivo efficacy of **50** was examined using db/db mice undergoing daily oral administration for 7 days.[36] As shown in Figure 5A, **50** dose-dependently lowered plasma glucose levels and exhibited excellent efficacy in db/db mice. Compound **50** exhibited 56% and 77% glucose normalization at doses of 30 and 100 mg/kg, respectively, compared with lean control animals. These responses are equivalent to the effects exhibited by 3 and 10 mg/kg doses, respectively, of rosiglitazone. Partial activation of PPARγ probably brings about the modest potency of **50** relative to the PPARγ full agonist, rosiglitazone.[37]

Some PPARγ partial agonists have been reported to cause no weight gain in db/db mice.[38] In contrast, some reports indicate that weight gain has been observed, and that even the tested compounds showed a decreased induction of adipogenesis in cells.[39] Unfortunately, there was no significant difference in the weight gain profiles between **50** and rosiglitazone after 7-day treatment of db/db mice (Figure 5B).

Table 3. Species Difference of **50** in the Transactivation of PPARγ Isoform

	transactivation[a]		
species	EC_{50} (μM)	efficacy (%)	IC_{50} (μM)
human	0.108	59	1.79
mice	0.158	59	1.77
rat	0.158	45	0.539

[a] Compounds were screened for agonist or antagonist activity on PPARγ-GAL4 chimeric receptors in transiently transfected CHO-K1 cells in the absence or presence of 1 μM rosiglitazone.

Conclusion

In conclusion, we have succeeded in the design, synthesis, and evaluation of 2,4,6-trisubstitutedpyrimidine-5-carboxylic acid derivatives based on the in-house screening hit of compound **7**. Structure−activity relationship studies have revealed that (1) the maximum activation level for PPARγ agonism can be adjusted by modulation of the length of the alkyl group at the 2-position on pyrimidine; (2) sulfur atoms at the 4- and 6-positions contribute to the potency and chemical properties, including water solubility. Among the synthesized pyrimidine-5-carboxylic acid derivatives, **50** was selected for further evaluation. The X-ray cocrystal structure of **50** on PPARγ revealed that the key hydrogen bond interactions, which are not related to the AF-2 site, are different from those of the full agonist. Pharmacological study of **50** demonstrated its excellent antidiabetic activity in db/db mice. These results indicate that **50** is attractive candidate as a new lead compound for design of more potent PPARγ partial agonist.

Experimental Section

Chemistry. Melting points were determined with a Yamato MP-500 melting point apparatus and are uncorrected. ^{1}H NMR

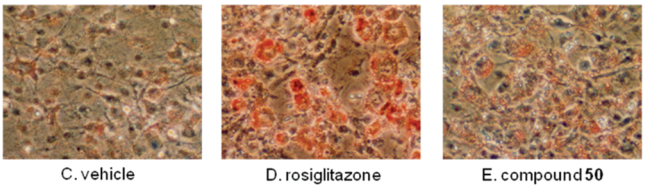


Figure 4. Visualization of lipid accumulation in 3T3-L1 cells by oil red O staining. After reaching confluence, cells were stimulated with 1 μM dexamethasone, 1 μM IBMX, and 2 μM insulin for 7 days. The DEX/MIX medium was then removed, and cells were treated for 10 days with the following ligands: C, vehicle; D, rosiglitazone; E, compound **50**.

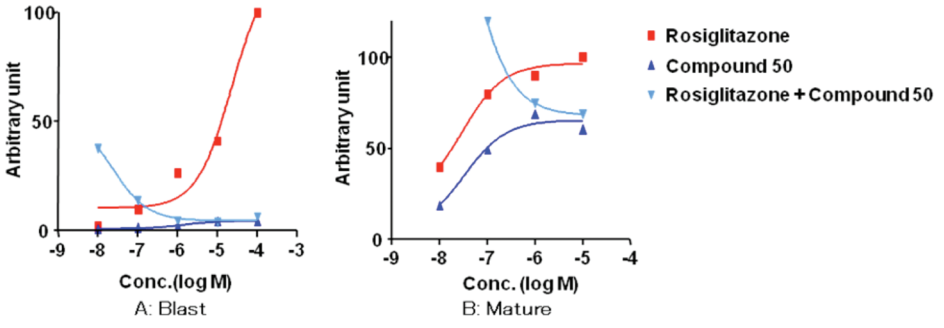


Figure 3. Effects of rosiglitazone and **50** on the differentiation of 3T3-L1 cells. Total RNA was extracted after 7 days for A, and after 10 days for B. Reverse transcription and real-time PCR were on cDNAs with an aP2-specific sample. One microgram of total RNA was used in each case.

Table 4. Pharmacokinetic Profiles of **50** in Mice

$C_{10\ min}$ (μg/mL)[a]	CL (mL/mim/kg)[a]	vd (L/kg)[a]	$t_{1/2}$ (h)[a]	AUC iv (μg*h/mL)[a]	AUC po (μg*h/mL)[b]	*F* (%)
6.89	1.88	0.12	0.76	8.86	3.17	35.8

[a] These values were measured in mice after iv administration (1 mg/kg). [b] These values were measured in mice after po administration (1 mg/kg).

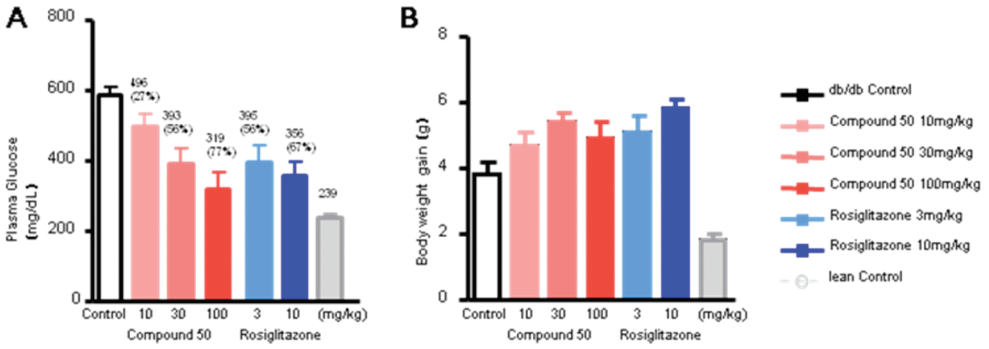


Figure 5. Effects of rosiglitazone and compound **50** with oral administration in db/db mice after 7 days. Data are shown as mean ± SEM $n = 5$. (A) Plasma glucose. (B) Body weight gain.

spectra were measured in $CDCl_3$ or DMSO-d_6 with TMS and the solvent peak as internal standards, on a JEOL ECA-400 (400 MHz) spectrometer. Mass spectra (MS) were obtained on a Hitachi M-2000 mass spectrometer. Column chromatography was carried out on Merck silica gel 60. Analytical thin-layer chromatography (TLC) was performed on Merck precoated silica gel 60F254 plates, and the compounds were visualized by UV illumination (254 nm) or by heating after spraying with phosphomolybdic acid in ethanol. The data for elemental analysis are within ±0.4% of theoretical values and were determined by a Yanaco CHN corder MT-5. All tested compounds had a purity of at least 95%.

Methyl 4-(Benzylthio)-6-chloro-2-(methylthio)pyrimidine-5-carboxylate (12). To a solution of **11**[23] (17.6 g, 69.5 mmol) in DMF (100 mL) were added potassium carbonate (9.70 g, 70.2 mmol) and phenymethanethiol (8.68 g, 69.9 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with ethyl acetate, then washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (hexane/AcOEt = 5:1) of the residue gave **12** (22.9 g, 97%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.56 (3H, s), 3.92 (3H, s), 4.44 (2H, s), 7.24−7.40 (5H, m). HRMS (EI) calcd for $C_{14}H_{13}ClN_2O_2S_2$ (M^+) 340.0107, found 340.0117.

Methyl 4-(Benzylamino)-6-chloro-2-(heptylamino)pyrimidine-5-carboxylate (16). To a solution of **14**[23] (130 mg, 0.365 mmol) in toluene (0.5 mL) was added a mixture of triethylamine (20 μL) and heptylamine (42.1 mg, 0.365 mmol) in toluene (0.5 mL) portion-wise under ice cooling. The mixture was stirred at 0 °C for 5 h. The resulting mixture was diluted with ethyl acetate and then washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 5:1) of the residue gave **16** (74.1 mg, 52%) as a colorless foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 6.7 Hz), 1.20−1.40 (8H, m), 1.45−1.55 (2H, m), 3.30−3.45 (2H, m), 3.84 (3H, s), 4.60−4.72 (2H, m), 5.05−5.33 (1H, br), 7.22−7.37 (5H, m), 8.58−8.97 (1H, br). HRMS (FAB^+) calcd for $C_{20}H_{27}ClN_4O_2$ ($M + H^+$) 390.1823, found 390.1816.

2-(Butylthio)-4,6-dichloro-pyrimidine (20). To a mixture of thiobarbituric acid (5.22 g, 36.2 mmol) in EtOH (50 mL) was added 3 N NaOH (12 mL) at room temperature, and the mixture was stirred under reflux for 1 h. The mixture was added iodobutane (4.20 mL, 36.9 mmol) at room temperature, and the mixture was stirred under reflux for 2 h. The mixture was cooled to room temperature. The precipitate was formed and collected by filtration, and then dried in vacuo. The mixture of the cake with phosphoryloxy chloride (15.0 mL, 0.161 mol) and *N,N*-dimethylaniline (5.2 mL, 41.0 mmol) was refluxed for 1 h. The mixture was poured into ice−water and extracted with ethyl acetate, then dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (hexane/AcOEt = 30:1) of the residue gave **20** (1.61 g, 19%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (3H, t, J = 7.3 Hz), 1.48 (2H, qt, J = 7.3 and 7.3 Hz), 1.71 (2H, tt, J = 7.3 and 7.3 Hz), 3.15 (2H, t, J = 7.3 Hz), 7.01 (1H, s). HRMS (FAB^+) calcd for $C_8H_{11}Cl_2N_2S$ ($M + H^+$) 237.0020, found 236.9995.

4,6-Dichloro-2-(octylthio)pyrimidine (21). The title compound **21** (696 mg, 7%) was prepared from thiobarbituric acid (5.22 g, 36.2 mmol) and iodooctane (6.30 mL, 36.2 mmol) in the same manner as described for **20**. Pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.3 Hz), 1.21−1.38 (8H, m), 1.38−1.49 (2H, m), 1.72 (2H, tt, J = 7.3 and 7.3 Hz), 3.14 (2H, t, J = 7.3 Hz), 7.01 (1H, s). HRMS (FAB^+) calcd for $C_{12}H_{19}Cl_2N_2S$ ($M + H^+$) 293.0646, found 293.0623.

2-(Butylthio)-4,6-dichloropyrimidine-5-carboxylic Acid (22). A 2.6 M solution of *n*-butyllithium (4.0 mL, 10.4 mmol) in hexanes was added to a solution of diisopropylamine (1.50 mL, 10.6 mmol) in THF (10 mL) at −10 °C under Ar. After the solution was stirred for 30 min, a solution of **20** (1.59 g, 6.70 mmol) in THF (10 mL) was added to the LDA solution at −78 °C. The mixture was stirred at the same temperature for 5 h, and CO_2 gas was then introduced for 10 min. After the reaction was quenched by the addition of water (10 mL) and 6 N HCl (10 mL), the mixture was extracted with ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. The residue was recrystallized from MeOH to afford **22** (1.40 g, 74%) as a pale yellow solid. Mp: 210−215 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.91 (3H, t, J = 7.3 Hz), 1.40 (2H, qt, J = 7.3 and 7.3 Hz), 1.63 (2H, tt, J = 7.3 and 7.3 Hz), 3.10 (2H, t, J = 7.3 Hz). HRMS (EI) calcd for $C_9H_{10}Cl_2N_2O_2S$ (M^+) 279.9840, found 279.9844. Anal. ($C_9H_{10}Cl_2N_2O_2S$ 2.2H_2O) C, H, N.

2-(Butylthio)-4,6-dichloropyrimidine-5-carboxylic Acid (23). The title compound **23** (420 mg, 54%) was prepared from **21** (680 mg, 2.32 mmol) in the same manner as described for **22**. Pale yellow solid. Mp: 210−214 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86 (3H, t, J = 7.3 Hz), 1.20−1.43 (10H, m), 1.65 (2H, tt, J = 7.3 and 7.3 Hz), 3.09 (2H, t, J = 7.3 Hz). HRMS (EI) calcd for $C_{13}H_{18}Cl_2N_2O_2S$ (M^+) 336.0466, found 336.0446. Anal. ($C_{13}H_{18}Cl_2N_2O_2S$ 2H_2O) C, H, N.

Methyl 2-(Butylthio)-4,6-dichloropyrimidine-5-carboxylate (24). To a solution of **22** (1.21 g, 4.30 mmol) in DMF (5 mL) were added potassium carbonate (595 mg, 4.31 mmol) and iodomethane

(0.30 mL, 4.82 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with ethyl acetate, then washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (hexane/AcOEt = 5:1) of the residue gave **24** (1.00 g, 66%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (3H, t, J = 7.3 Hz), 1.47 (2H, qt, J = 7.3 and 7.3 Hz), 1.72 (2H, tt, J = 7.3 and 7.3 Hz), 3.16 (2H, t, J = 7.3 Hz), 3.98 (3H, s). HRMS (EI) calcd for $C_{10}H_{12}Cl_2N_2O_2S$ (M^+) 293.9997, found 293.9977.

Methyl 4,6-Dichloro-2-(octylthio)pyrimidine-5-carboxylate (25). The title compound **25** (400 mg, 86%) was prepared from **23** (445 mg, 1.32 mmol) and iodomethane (0.10 mL, 1.61 mmol) in the same manner as described for **24**. Pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.3 Hz), 1.20−1.37 (8H, m), 1.37−1.49 (2H, m), 1.72 (2H, tt, J = 7.3 and 7.3 Hz), 3.15 (2H, t, J = 7.3 Hz), 3.98 (3H, s). HRMS (EI) calcd for $C_{14}H_{20}Cl_2N_2O_2S$ (M^+) 350.0623, found 350.0657.

Methyl 4-(Benzylthio)-2-(butylthio)-6-chloropyrimidine-5-carboxylate (26). To a solution of **24** (96.0 mg, 0.325 mmol) in DMF (1 mL) were added potassium carbonate (45.0 mg, 0.326 mmol) and phenyl methanethiol (41.0 mg, 0.330 mmol) at 0 °C, and the mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with ethyl acetate, then washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (hexane/AcOEt = 5:1) of the residue gave **26** (107 mg, 86%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (3H, t, J = 7.3 Hz), 1.45 (2H, qt, J = 7.3 and 7.3 Hz), 1.71 (2H, tt, J = 7.3 and 7.3 Hz), 3.13 (2H, t, J = 7.3 Hz), 3.92 (3H, s), 4.43 (2H, s), 7.24−7.40 (5H, m). HRMS (FAB$^+$) calcd for $C_{17}H_{20}ClN_2O_2S_2$ ($M + H^+$) 383.0655, found 383.0696.

Methyl 4-(Benzylthio)-6-chloro-2-(octylthio)pyrimidine-5-carboxylate (27). The title compound **27** (110 mg, 70%) was prepared from **25** (126 mg, 0.359 mmol) and phenyl methanethiol (45.0 mg, 0.362 mmol) in the same manner as described for **26**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (3H, t, J = 7.3 Hz), 1.18−1.35 (8H, m), 1.35−1.45 (2H, m), 1.66−1.76 (2H, m), 3.12 (2H, t, J = 7.3 Hz), 3.92 (3H, s), 4.43 (2H, s), 7.21−7.40 (5H, m). HRMS (EI) calcd for $C_{21}H_{27}ClN_2O_2S_2$ (M^+) 438.1202, found 438.1167.

4,6-Dichloropyrimidine-5-carboxylic Acid (29). Step 1. 4,6-Dichloropyrimidine-5-carboxaldehyde. To a mixture of phosphoryl oxychloride (8.42 mL, 89.2 mmol) and DMF (2.76 mL, 35.7 mmol), 4,6-dihydroxypyrimidine (2.00 g, 17.8 mmol) was added over 40 min and then stirred at 100 °C for 5 h. The mixture was poured into ice−water and then extracted with Et_2O. The organic layer was washed with H_2O and dried over Na_2SO_4, then concentrated in vacuo. The compound **29 Step 1** (2.04 g, 65%) was given as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (1H, s), 10.46 (1H, s).

Step 2. 4,6-Dichloropyrimidine-5-carboxylic acid (**29**). To a mixture of the compound of step 1 (2.00 g, 11.3 mmol), 2-methyl-2-butene (6.00 mL, 56.6 mmol), NaH_2PO_4 (6.14 g, 51.2 mmol), *t*-BtOH (80 mL), and H_2O (20 mL) was added $NaClO_2$ (4.09 g, 45.2 mmol) and then stirred at 0 °C for 4 h. After being warmed at room temperature, the mixture was diluted with AcOEt, washed with H_2O and brine, and dried over Na_2SO_4. The title compound **29** (1.28 g, 59%) was given as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (1H, s).

Methyl 4,6-Dichloropyrimidine-5-carboxylate (30). To a solution of **29** (386 mg, 2.00 mmol) in DMF (2 mL) were added potassium carbonate (277 mg, 2.00 mmol) and iodomethane (0.15 mL, 2.41 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with ethyl acetate, then washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (hexane/AcOEt = 5:1) of the residue gave **30** (217 mg, 52%) as a pale yellow solid. Mp: 47−49 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.03 (3H, s), 8.84 (1H, s). HRMS (FAB$^+$) calcd for $C_6H_5Cl_2N_2O_2$ ($M + H^+$) 206.9728, found 206.9736. Anal. ($C_6H_4Cl_2N_2O_2$ $0.3H_2O$) C, H, N.

Methyl 4-(Benzylthio)-6-chloropyrimidine-5-carboxylate (31). To a solution of methyl **30** (115 mg, 0.556 mmol) in DMF (1 mL) were added potassium carbonate (77.0 mg, 0.557 mmol) and phenyl methanethiol (70.0 mg, 0.564 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with ethyl acetate, then washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (hexane/AcOEt = 5:1) of the residue gave **31** (147 mg, 90%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 (3H, s), 4.48 (2H, s), 7.24−7.35 (3H, m), 7.38 (2H, d, J = 6.8 Hz), 8.75 (1H, s). HRMS (FAB$^+$) calcd for $C_{13}H_{12}ClN_2O_2S$ ($M + H^+$) 295.0308, found 295.0297.

4-(Benzylamino)-6-(benzylthio)-2-(methylthio)pyrimidine-5-carboxylic Acid (9). Step 1. Methyl 4-(benzylamino)-6-(benzylthio)-2-(methylthio)pyrimidine-5-carboxylate. To a solution of **12** (100 mg, 0.309 mmol) in DMF (1 mL) were added potassium carbonate (85.5 mg, 0.619 mmol) and phenyl methanethiol (42.5 mg, 0.342 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with ethyl acetate, then washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 5:1) of the residue gave **9 Step 1** as a pale yellow solid (126 mg, 99%). Mp: 91−93 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.46 (3H, s), 3.84 (3H, s), 4.37 (2H, s), 4.74 (2H, d, J = 5.5 Hz), 7.20−7.37 (8H, m), 7.39 (2H, d, J = 7.3 Hz), 8.90 (1H, t, J = 5.5 Hz). HRMS (FAB$^+$) calcd for $C_{21}H_{22}N_3O_2S_2$ ($M + H^+$) 412.1153, found 412.1112. Anal. ($C_{21}H_{21}N_3O_2S_2$ $0.2H_2O$) C, H, N.

Step 2. 4-(Benzylamino)-6-(benzylthio)-2-(methylthio)pyrimidine-5-carboxylic acid (**9**). To a solution of the compound of Step 1 (108 mg, 0.262 mmol) in THF-MeOH (1.5 mL; 1:2 v/v) was added 3 N KOH (0.5 mL) at room temperature, and the mixture was refluxed for 4 h. The resulting mixture was cooled to room temperature, and water and 2 N HCl (1 mL) were then added. The precipitate was formed and collected by filtration, and dried in vacuo. **9** was given as a colorless solid (80.0 mg, 77%). Mp: 165−167 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.39 (3H, s), 4.28 (2H, s), 4.68 (2H, d, J = 6.1 Hz), 7.20−7.42 (10H, m), 8.98−9.10 (1H, br), 13.65−14.05 (1H, br). HRMS (EI) calcd for $C_{20}H_{19}N_3O_2S_2$ (M^+) 397.0919, found 397.0944. Anal. ($C_{20}H_{19}N_3O_2S_2$ $0.1H_2O$) C, H, N.

4-Anilino-6-(benzylthio)-2-(methylthio)pyrimidine-5-carboxylic Acid (32). Step 1. Methyl 4-anilino-6-(benzylthio)-2-(methylthio)-pyrimidine-5-carboxylate. The compound of Step 1 (35.6 mg, 28%) was prepared from **12** (100 mg, 0.323 mmol) and phenyl methanethiol (45.0 mg, 0.362 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. Mp: 102−103 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (3H, s), 3.92 (3H, s), 4.39 (2H, s), 7.12 (1H, d, J = 7.3 Hz), 7.22−7.37 (5H, m), 7.40 (2H, d, J = 7.3 Hz), 7.62 (2H, d, J = 7.3 Hz), 10.70 (1H, s). HRMS (FAB$^+$) calcd for $C_{20}H_{20}N_3O_2S_2$ ($M + H^+$) 398.0997, found 398.1002. Anal. ($C_{20}H_{19}N_3O_2S_2$) C, H, N.

Step 2. 4-Anilino-6-(benzylthio)-2-(methylthio)pyrimidine-5-carboxylic acid (**32**). The title compound **32** (26.5 mg, 92%) was prepared from the compound of Step 1 (30.0 mg, 75.5 μmol) in the same manner as described for **9**. Colorless solid. Mp: 192−195 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.48 (3H, s), 4.33 (2H, s), 7.09−7.16 (1H, m), 7.22−7.29 (1H, m), 7.29−7.45 (6H, m), 7.61 (2H, dd, J = 8.5 and 1.2 Hz), 10.74−10.85 (1H, br), 13.95−14.75 (1H, br). HRMS (FAB$^+$) calcd for $C_{19}H_{18}N_3O_2S_2$ ($M^+ + 1$) 384.0840, found 384.0851. Anal. ($C_{19}H_{17}N_3O_2S_2$ $0.2H_2O$) C, H, N.

4-(Benzylthio)-2-(methylthio)-6-(2-phenethylamino)pyrimidine-5-carboxylic Acid (33). Step 1. Methyl 4-(benzylthio)-2-(methylthio)-6-(phenethylamino)pyrimidine-5-carboxylate. The compound of Step 1 (38.7 mg, 53%) was prepared from **12** (57.4 mg, 0.170 mmol) and phenyl methanethiol (23.2 mg, 0.359 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. Mp:

152−153 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.53 (3H, s), 2.92 (2H, t, J = 7.3 Hz), 3.77 (2H, dd, J = 7.3 and 5.5 Hz), 3.81 (3H, s), 4.37 (2H, s), 7.20−7.35 (8H, m), 7.39 (2H, d, J = 7.3 Hz), 8.51 (1H, t, J = 5.5 Hz). HRMS (FAB$^+$) calcd for $C_{22}H_{24}N_3O_2S_2$ ($M + H^+$) 426.1310, found 426.1327. Anal. ($C_{22}H_{23}N_3O_2S_2$ 0.1H_2O) C, H, N.

Step 2. 4-(Benzylthio)-2-(methylthio)-6-(2-phenethylamino)-pyrimidine-5-carboxylic acid (**33**). The title compound **33** (29.7 mg, 93%) was prepared from the compound of Step 1 (33.0 mg, 77.5 μmol) in the same manner as described for **9**. Colorless solid. Mp: 175−177 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (3H, s), 2.85 (2H, t, J = 6.7 Hz), 3.62−3.72 (2H, m), 4.28 (2H, s), 7.18−7.42 (10H, m), 8.65−8.77 (1H, br), 13.50−14.05 (1H, br). HRMS (FAB$^+$) calcd for $C_{21}H_{22}N_3O_2S_2$ ($M^+ + 1$) 412.1153, found 412.1176. Anal. ($C_{21}H_{21}N_3O_2S_2$) C, H, N.

4-(Benzylthio)-2-(methylthio)-6-(2-pyridylmethylamino)pyrimidine-5-carboxylic Acid (34). Step 1. Methyl 4-(benzylthio)-2-(methylthio)-6-(2-pyridylmethylamino)pyrimidine-5-carboxylate. The compound of Step 1 (87.5 mg, 85%) was prepared from **12** (85.3 mg, 0.250 mmol)and 2-pyridylmethylamine (30.0 mg, 0.277 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. mp: 107−109 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (3H, s), 3.90 (3H, s), 4.38 (2H, s), 4.85 (2H, d, J = 5.5 Hz), 7.17−7.34 (5H, m), 7.39 (2H, d, J = 7.3 Hz), 7.65 (1H, td, J = 7.3 and 1.8 Hz), 8.60 (1H, dd, J = 3.1 and 1.8 Hz), 9.34 (1H, t, J = 5.5 Hz). HRMS (FAB$^+$) calcd for $C_{20}H_{21}N_4O_2S_2$ ($M + H^+$) 413.1106, found 413.1129. Anal. ($C_{20}H_{20}N_4O_2S_2$ 0.2H_2O) C, H, N.

Step 2. 4-(Benzylthio)-2-(methylthio)-6-(2-pyridylmethylamino)pyrimidine-5-carboxylic Acid (**34**). The title compound **34** (60.0 mg, 82%) was prepared from the compound of Step 1 (76.0 mg, 0.184 mmol) in the same manner as described for **9**. Colorless solid. Mp: 192−194 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.36 (3H, s), 4.28 (2H, s), 4.76 (2H, d, J = 4.9 Hz), 7.20−7.35 (5H, m), 7.35−7.42 (2H, m), 7.75 (1H, td, J = 7.3 and 1.2 Hz), 8.52 (1H, dd, J = 4.9 and 1.8 Hz), 9.27−9.42 (1H, br), 13.50−14.10 (1H, br). HRMS (FAB$^+$) calcd for $C_{19}H_{19}N_4O_2S_2$ ($M^+ + 1$) 399.0949, found 399.0952. Anal. ($C_{19}H_{18}N_4O_2S_2$ 0.1H_2O) C, H, N.

4-(Benzylthio)-2-(methylthio)-6-(3-pyridylmethylamino)pyrimidine-5-carboxylic Acid (35). Step 1. Methyl 4-(benzylthio)-2-(methylthio)-6-(3-pyridylmethylamino)pyrimidine-5-carboxylate. The compound of Step 1 (91.0 mg, 74%) was prepared from **12** (102 mg, 0.299 mmol) and 3-pyridylmethylamine (34.0 mg, 0.314 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. Mp: 121−123 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (3H, s), 3.86 (3H, s), 4.37 (2H, s), 4.75 (2H, d, J = 6.1 Hz), 7.21−7.28 (2H, m), 7.31 (2H, t, J = 7.3 Hz), 7.39 (2H, d, J = 7.3 Hz), 7.64 (1H, d, J = 7.3 Hz), 8.52 (1H, d, J = 3.1 Hz), 8.59 (1H, d, J = 1.2 Hz), 8.99 (1H, t, J = 6.1 Hz). HRMS (FAB$^+$) calcd for $C_{20}H_{21}N_4O_2S_2$ ($M + H^+$) 413.1106, found 413.1149. Anal. ($C_{20}H_{20}N_4O_2S_2$) C, H, N.

Step 2. 4-(Benzylthio)-2-(methylthio)-6-(3-pyridylmethylamino)pyrimidine-5-carboxylic Acid (**35**). The title compound **35** (25.0 mg, 35%) was prepared from the compound of Step 1 (73.0 mg, 0.177 mmol) in the same manner as described for **9**. Colorless solid. Mp: 197−200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.38 (3H, s), 4.27 (2H, s), 4.69 (2H, d, J = 5.5 Hz), 7.20−7.40 (6H, m), 7.70 (1H, dt, J = 7.9 and 1.8 Hz), 8.44 (1H, dd, J = 4.9 and 1.8 Hz), 8.54 (1H, d, J = 1.8 Hz), 9.09−9.20 (1H, br). HRMS (EI) calcd for $C_{19}H_{18}N_4O_2S_2$ (M^+) 398.0871, found 398.0841. Anal. ($C_{19}H_{18}N_4O_2S_2$ 0.3H_2O) C, H, N.

4-(Benzylthio)-2-(methylthio)-6-(4-pyridylmethylamino)pyrimidine-5-carboxylic Acid (36). Step 1. Methyl 4-(benzylthio)-2-(methylthio)-6-(4-pyridylmethylamino)pyrimidine-5-carboxylate. The compound of Step 1 (81.3 mg, 79%) was prepared from **12** (85.3 mg, 0.250 mmol) and 4-pyridylmethylamine (30.0 mg, 0.277 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. Mp: 111−114 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.37 (3H, s), 3.88 (3H, s), 4.37 (2H, s), 4.75 (2H, d, J = 6.1 Hz), 7.18−7.27 (4H, m), 7.31 (2H, t, J = 6.7 Hz), 7.40 (2H, d, J = 6.7 Hz), 8.52−8.57 (2H, m), 9.05 (1H, t, J = 6.1 Hz). HRMS (FAB$^+$) calcd for $C_{20}H_{21}N_4O_2S_2$ ($M + H^+$) 413.1106, found 413.1131. Anal. ($C_{20}H_{20}N_4O_2S_2$ 0.2H_2O) C, H, N.

Step 2. 4-(Benzylthio)-2-(methylthio)-6-(4-pyridylmethylamino)pyrimidine-5-carboxylic acid (**36**). The title compound **36** (62.5 mg, 92%) was prepared from the compound of Step 1 (70.0 mg, 0.170 mmol) in the same manner as described for **9**. Colorless solid. Mp: 202−203 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.30 (3H, s), 4.26 (2H, s), 4.69 (2H, d, J = 5.5 Hz), 7.20−7.32 (5H, m), 7.35−7.40 (2H, m), 8.47 (2H, dd, J = 4.3 and 1.8 Hz), 9.15−9.32 (1H, br). HRMS (FAB$^+$) calcd for $C_{19}H_{19}N_4O_2S_2$ ($M^+ + 1$) 399.0949, found 399.0941. Anal. ($C_{19}H_{18}N_4O_2S_2$ 0.6H_2O) C, H, N.

4-(Benzylthio)-6-(methylamino)-2-(methylthio)pyrimidine-5-carboxylic Acid (37). Step 1. Methyl 4-(benzylthio)-6-(methylamino)-2-(methylthio)pyrimidine-5-carboxylate. The compound of Step 1 (83.2 mg, 83%) was prepared from methyl **12** (102 mg, 0.300 mmol) and methylamine (0.2 mL, 2 M solution in THF, 0.4 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. Mp: 151−153 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.53 (3H, s), 3.05 (3H, d, J = 4.9 Hz), 3.85 (3H, s), 4.38 (2H, s), 7.22−7.26 (1H, m), 7.31 (2H, t, J = 7.3 Hz), 7.39 (2H, d, J = 7.3 Hz), 8.42−8.50 (1H, br). HRMS (EI) calcd for $C_{15}H_{17}N_3O_2S_2$ (M^+) 335.0762, found 335.0739. Anal. ($C_{15}H_{17}N_3O_2S_2$) C, H, N.

Step 2. 4-(Benzylthio)-6-(methylamino)-2-(methylthio)pyrimidine-5-carboxylic acid (**37**). The title compound **37** (61.5 mg, 91%) was prepared from the compound of Step 1 (70.7 mg, 0.211 mmol) in the same manner as described for **9**. Colorless solid. Mp: 169−171 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.48 (3H, s), 2.93 (3H, d, J = 3.7 Hz), 4.29 (2H, s), 7.23 (1H, t, J = 7.3 Hz), 7.30 (2H, t, J = 7.3 Hz), 7.37 (2H, d, J = 7.3 Hz), 8.50 (1H, brs), 13.72 (1H, brs). HRMS (EI) calcd for $C_{14}H_{15}N_3O_2S_2$ (M^+) 321.0606, found 321.0579. Anal. ($C_{14}H_{15}N_3O_2S_2$) C, H, N.

4-(Benzylthio)-2-(methylthio)-4-(propylamino)pyrimidine-5-carboxylic Acid (38). Step 1. Methyl 4-(benzylthio)-2-(methylthio)-4-(propylamino)pyrimidine-5-carboxylate. The compound of Step 1 (82.8 mg, 76%) was prepared from **12** (102 mg, 0.300 mmol) and propylamine (20.0 mg, 0.338 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. Mp: 94−96 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.3 Hz), 1.58−170 (3H, m), 2.50 (3H, s), 3.45−3.53 (2H, m), 3.85 (3H, s), 4.37 (2H, s), 7.21−7.27 (1H, m), 7.30 (2H, t, J = 7.3 Hz), 7.39 (2H, d, J = 7.3 Hz), 8.55−8.65 (1H, br). HRMS (EI) calcd for $C_{17}H_{21}N_3O_2S_2$ (M^+) 363.1075, found 363.1060. Anal. ($C_{17}H_{21}N_3O_2S_2$) C, H, N.

Step 2. 4-(Benzylthio)-2-(methylthio)-4-(propylamino)pyrimidine-5-carboxylic acid (**38**). The title compound **38** (60.0 mg, 92%) was prepared from the compound of Step 1 (68.0 mg, 0.187 mmol) in the same manner as described for **9**. Colorless solid. Mp: 159−162 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (3H, t, J = 7.3 Hz), 1.50−1.61 (2H, m), 2.47 (3H, s), 3.37−3.45 (2H, m), 4.28 (2H, s), 7.23 (1H, t, J = 7.3 Hz), 7.30 (2H, t, J = 7.3 Hz), 7.37 (2H, d, J = 7.3 Hz), 8.60−8.68 (1H, br), 13.78 (1H, brs). HRMS (EI) calcd for $C_{16}H_{19}N_3O_2S_2$ (M^+) 349.0919, found 349.0922. Anal. ($C_{16}H_{19}N_3O_2S_2$) C, H, N.

4-(Benzylthio)-6-(cyclopropylamino)-2-(methylthio)pyrimidine-5-carboxylic Acid (39). Step 1. Methyl 4-(benzylthio)-6-(cyclopropyl)-2-(methylthio)pyrimidine-5-carboxylate. The compound of Step 1 (64.8 mg, 60%) was prepared from **12** (102 mg, 0.299 mmol) and cyclopropylamine (19.0 mg, 0.333 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. Mp: 107−110 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.52−0.58 (2H, m), 0.79−0.85 (2H, m), 2.54 (3H, s), 2.92−3.00 (1H, m), 3.84 (3H, s), 4.37 (2H, s), 7.20−7.27 (1H, m), 7.30 (2H, t, J = 7.3 Hz), 7.39 (2H, d, J = 7.3 Hz), 8.51 (1H, brs). HRMS (EI) calcd for $C_{17}H_{19}N_3O_2S_2$ (M^+) 361.0919, found 361.0879. Anal. ($C_{17}H_{19}N_3O_2S_2$) C, H, N.

Step 2. 4-(Benzylthio)-6-(cyclopropylamino)-2-(methylthio)-pyrimidine-5-carboxylic acid (**39**). The title compound **39** (52.0 mg, 95%) was prepared from the compound of Step 1 (57.0 mg, 0.158 mmol) in the same manner as described for **9**. Colorless solid. Mp:

177−180 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.50−0.56 (2H, m), 0.72−0.80 (2H, m), 2.87−2.96 (1H, m), 4.30 (2H, s), 7.23 (1H, t, J = 7.3 Hz), 7.30 (2H, t, J = 7.3 Hz), 7.37 (2H, d, J = 7.3 Hz), 8.50−8.60 (1H, br), 13.65−14.00 (1H, br). HRMS (FAB$^+$) calcd for $C_{16}H_{18}N_3O_2S_2$ (M^+ + 1) 348.0840, found 348.0850. Anal. ($C_{16}H_{17}N_3O_2S_2$ 0.2H_2O) C, H, N.

4-(Benzylthio)-6-(cyclohexylamino)-2-(methylthio)pyrimidine-5-carboxylic Acid (40). Step 1. Methyl 4-(benzylthio)-6-(cyclohexylamino)-2-(methylthio)pyrimidine-5-carboxylate. The compound of Step 1 (110 mg, 43%) was prepared from **12** (208 mg, 0.610 mmol) and cyclohexylamine (80 μL, 0.615 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. Mp: 76−79 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92−1.04 (2H, m), 1.14−1.30 (4H, m), 1.62−1.82 (4H, m), 2.50 (3H, s), 3.37 (2H, t, J = 6.1 Hz), 3.85 (2H, s), 4.36 (2H, s), 7.21−7.27 (1H, m), 7.31 (2H, d, J = 7.3 Hz), 7.37 (2H, d, J = 7.3 Hz), 8.66 (1H, t, J = 6.1 Hz). HRMS (EI) calcd for $C_{21}H_{27}N_3O_2S_2$ (M^+) 417.1545, found 417.1592. Anal. ($C_{21}H_{27}N_3O_2S_2$) C, H, N.

Step 2. 4-(Benzylthio)-6-(cyclohexylamino)-2-(methylthio)-pyrimidine-5-carboxylic acid (**40**). The title compound **40** (80.1 mg, 93%) was prepared from the compound of Step 1 (89.3 mg, 0.214 mmol) in the same manner as described for **9**. Colorless solid. Mp: 168−170 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87−1.00 (2H, m), 1.05−1.25 (3H, m), 1.48−1.72 (6H, m), 2.46 (3H, s), 2.40−2.55 (2H, m), 4.28 (2H, s), 7.20−7.26 (1H, m), 7.26−7.32 (2H, m), 7.32−7.42 (2H, m), 8.70 (1H, t, J = 5.5 Hz), 13.50−14.10 (1H, br). HRMS (FAB$^+$) calcd for $C_{20}H_{26}N_3O_2S_2$ (M^+ + 1) 404.1466, found 404.1484. Anal. ($C_{20}H_{25}N_3O_2S_2$) C, H, N..

4-(Benzylamino)-6-(benzylthio)-2-(butylthio)pyrimidine-5-carboxylic Acid (41). Step 1. Methyl 4-(benzylamino)-6-(benzylthio)-2-(butylthio)pyrimidine-5-carboxylate. The compound of Step 1 (95.0 mg, 98%) was prepared from **26** (82.0 mg, 0.214 mmol) and benzylamine (27.5 mg, 0.257 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. Mp: 87−89 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.3 Hz), 1.32−1.44 (2H, m), 1.60−1.70 (2H, m), 3.04 (2H, t, J = 7.3 Hz), 3.84 (3H, s), 4.36 (2H, s), 4.74 (2H, d, J = 5.5 Hz), 7.20−7.35 (8H, m), 7.39 (2H, d, J = 6.7 Hz), 8.89 (1H, t, J = 5.5 Hz). HRMS (EI) calcd for $C_{24}H_{27}N_3O_2S_2$ (M^+) 453.1545, found 453.1573. Anal. ($C_{24}H_{27}N_3O_2S_2$ 0.2H_2O) C, H, N.

Step 2. 4-(Benzylamino)-6-(benzylthio)-2-(butylthio)pyrimidine-5-carboxylic acid (**41**). The title compound **41** (55.0 mg, 74%) was prepared from the compound of Step 1 (77.0 mg, 0.170 mmol) in the same manner as described for **9**. Colorless solid. Mp: 156−158 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.80 (3H, t, J = 7.3 Hz), 1.28 (2H, qd, J = 7.3 and 7.3 Hz), 1.53 (2H, tt, J = 7.3 and 7.3 Hz), 2.96 (2H, t, J = 7.3 Hz), 4.27 (2H, s), 4.69 (2H, d, J = 6.1 Hz), 7.20−7.40 (10H, m), 13.40−14.20 (1H, br). HRMS (FAB$^+$) calcd for $C_{23}H_{26}N_3O_2S_2$ (M^+ + 1) 440.1466, found 440.1483. Anal. ($C_{23}H_{25}N_3O_2S_2$) C, H, N.

4-(Benzylamino)-6-(benzylthio)-2-(octylthio)pyrimidine-5-carboxylic Acid (42). Step 1. Methyl 4-(benzylamino)-6-(benzylthio)-2-(octylthio)pyrimidine-5-carboxylate. The compound of Step 1 (112 mg, 88%) was prepared from **27** (110 mg, 0.251 mmol) and benzylamine (32.0 mg, 0.299 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. Mp: 65−67 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (3H, t, J = 6.7 Hz), 1.18−1.40 (10H, m), 1.61−1.72 (2H, m), 3.03 (2H, t, J = 7.3 Hz), 3.84 (3H, s), 4.36 (2H, s), 4.74 (2H, d, J = 5.5 Hz), 7.21−7.41 (10H, m), 8.89 (1H, t, J = 5.5 Hz). HRMS (EI) calcd for $C_{28}H_{35}N_3O_2S_2$ (M^+) 509.2171, found 509.2138. Anal. ($C_{28}H_{35}N_3O_2S_2$ 0.25H_2O) C, H, N.

Step 2. 4-(Benzylamino)-6-(benzylthio)-2-(octylthio)pyrimidine-5-carboxylic acid (**42**). The title compound **42** (85.3 mg, 95%) was prepared from the compound of Step 1 (93.0 mg, 0.182 mmol) in the same manner as described for **9**. Colorless solid. Mp: 145−149 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (3H, t, J = 7.3 Hz), 1.10−1.30 (10H, m), 1.54 (2H, tt, J = 7.3 and 7.3 Hz), 2.96 (2H, t, J = 7.3 Hz), 7.20−7.40 (10H, m), 8.95−9.10 (1H, br), 13.50−14.20 (1H, br). HRMS (FAB$^+$) calcd for $C_{27}H_{34}N_3O_2S_2$ (M^+ + 1) 496.2092, found 496.2107. Anal. ($C_{27}H_{33}N_3O_2S_2$) C, H, N.

4-(Benzylamino)-6-(benzylthio)pyrimidine-5-carboxylic Acid (43). Step 1. Methyl 4-(benzylamino)-6-(benzylthio)pyrimidine-5-carboxylate. The compound of Step 1 (90.5 mg, 67%) was prepared from **31** (115 mg, 0.390 mmol) and benzylamine (50.0 mg, 0.467 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. Mp: 84−87 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (3H, s), 4.41 (2H, s), 4.76 (2H, d, J = 5.5 Hz), 7.21−7.36 (8H, m), 7.40 (2H, d, J = 6.8 Hz), 8.42 (1H, s), 8.83 (1H, t, J = 5.5 Hz). HRMS (EI) calcd for $C_{20}H_{19}N_3O_2S$ (M^+) 365.1198, found 365.1191. Anal. ($C_{20}H_{19}N_3O_2S$) C, H, N.

Step 2. 4-(Benzylamino)-6-(benzylthio)pyrimidine-5-carboxylic acid (**43**). The title compound **43** (63.0 mg, 91%) was prepared from the compound of Step 1 (72.0 mg, 0.197 mmol) in the same manner as described for **9**. Colorless solid. Mp: 223−226 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.32 (2H, s), 4.70 (2H, d, J = 6.1 Hz), 7.20−7.40 (10H, m), 8.39 (1H, s), 8.89 (1H, t, J = 6.1 Hz), 13.70−14.40 (1H, br). HRMS (FAB$^+$) calcd for $C_{19}H_{18}N_3O_2S$ (M^+ + 1) 352.1120, found 352.1156. Anal. ($C_{19}H_{17}N_3O_2S$) C, H, N.

4-(Benzylamino)-6-(benzyloxy)-2-(methylthio)pyrimidine-5-carboxylic Acid (44). Step 1. Methyl 4-(benzylamino)-6-(benzyloxy)-2-(methylthio)pyrimidine-5-carboxylate. The compound of Step 1 (165 mg, 90%) was prepared from **13** (150 mg, 0.463 mmol) and benzylalcohol (100 mg, 0.925 mmol) in the same manner as described for **9 Step 1**. Pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.46 (3H, s), 4.72 (2H, d, J = 6.1 Hz), 5.34 (2H, s), 7.25−7.45 (10H, m), 8.72 (1H, t, J = 6.1 Hz). HRMS (EI) calcd for $C_{21}H_{21}N_3O_3S$ (M^+) 395.1304, found 395.1306.

Step 2. 4-(Benzylamino)-6-(benzyloxy)-2-(methylthio)pyrimidine-5-carboxylic acid (**44**). The title compound **44** (61.8 mg, 80%) was prepared from the compound of Step 1 (80.0 mg, 0.202 mmol) in the same manner as described for **9**. Colorless solid. Mp: 108−112 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.32 (3H, s), 4.62 (2H, d, J = 5.5 Hz), 5.36 (2H, s), 7.13−7.33 (8H, m), 7.39 (2H, d, J = 7.3 Hz), 9.20 (1H, t, J = 5.5 Hz), 11.50−13.50 (1H, br). HRMS (FAB$^+$) calcd for $C_{20}H_{20}N_3O_3S$ (M^+ + 1) 382.1225, found 382.1191. Anal. ($C_{20}H_{19}N_3O_3S$ 0.5H_2O) C, H, N.

4-(Benzylamino)-6-(benzylthio)-2-(butylamino)pyrimidine-5-carboxylic Acid (45). Step 1. Methyl 4-(benzylamino)-6-(benzylthio)-2-(butylamino)pyrimidine-5-carboxylate. The compound of Step 1 (58.6 mg, 94%) was prepared from **15** (50.0 mg, 0.143 mmol) and phenyl methanethiol (21.5 mg, 0.173 mmol) in the same manner as described for **9 Step 1**. Pale yellow solid. Mp: 109−112 °C. ^{1}H NMR (400Mz, $CDCl_3$) δ 0.90 (3H, t, J = 7.3 Hz), 1.28−1.40 (2H, m), 1.46−1.58 (2H, m), 3.31−3.47 (2H, m), 3.80 (3H, s), 4.24−4.42 (2H, br), 4.61−4.75 (2H, br), 5.02−5.13 (1H, br), 7.20−7.42 (10H, m), 8.73−8.98 (1H, br).

Step 2. 4-(Benzylamino)-6-(benzylthio)-2-(butylamino)pyrimidine-5-carboxylic acid (**45**). The title compound **45** (38.0 mg, 83%) was prepared from the compound of Step 1 (47.6 mg, 0.109 mmol) in the same manner as described for **9**. Colorless solid. Mp: 145−147 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (3H, t, J = 7.3 Hz), 1.18−1.30 (2H, m), 1.33−1.50 (2H, m), 3.15−3.30 (3H, m), 4.22 and 4.30 (2H, each s), 4.63 (2H, d, J = 6.1 Hz), 7.18−7.42 (11H, m), 8.81 and 8.94 (1H, each t, J = 6.1 Hz), 12.70−13.10 (1H, br). HRMS (FAB$^+$) calcd for $C_{23}H_{27}N_4O_2S$ (M^+ + 1) 423.1855, found 423.1880. Anal. ($C_{23}H_{26}N_4O_2S$) C, H, N.

4-(Benzylamino)-6-(benzylthio)-2-(heptylamino)pyrimidine-5-carboxylic Acid (46). To a solution of **16** (68.0 mg, 0.174 mmol) in DMF (0.5 mL) were added potassium carbonate (72.1 mg, 0.522 mmol) and phenyl methanethiol (43.5 mg, 0.350 mmol) at room temperature, and the mixture was stirred at 80 °C for 3 h. To the resulting mixture was added 2 N HCl (1 mL) under ice cooling. The precipitate was formed and collected by filtration, and then dried in vacuo. The title compound **46** (48.7 mg, 60%)

was given as a colorless solid. Mp: 141−144 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.75−0.90 (3H, m), 1.12−1.32 (9H, m), 1.32−1.52 (2H, m), 3.12−3.23 (1H, m), 4.22 and 4.29 (2H, each s), 4.63 (2H, t, J = 5.5 Hz), 7.16−7.43 (11H, m), 8.80−9.00 (1H, br), 12.70−13.10 (1H, br). HRMS (FAB$^+$) calcd for $C_{26}H_{33}N_4O_2S$ ($M^+ + 1$) 465.2324, found 465.2351. Anal. ($C_{26}H_{32}N_4O_2S$ 0.57-H_2O) C, H, N.

4-(Benzylamino)-6-(benzylthio)-2-(*N*-butyl-*N*-methylamino)-pyrimidine-5-carboxylic Acid (47). The title compound **47** (19.0 mg, 39%) was prepared from **17** (40.4 mg, 0.111 mmol) and phenyl methanethiol (28.0 mg, 0.225 mmol) in the same manner as described for **46**. Colorless solid. Mp: 140−142 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.74−0.86 (3H, m), 1.08−1.22 (2H, m), 1.30−1.52 (2H, m), 2.96−3.12 (3H, m), 3.40−3.60 (2H, m), 4.26 (2H, s), 4.63 (2H, t, J = 5.5 Hz), 7.17−7.41 (10H, m), 8.85−9.00 (1H, br), 12.85−13.10 (1H, br). HRMS (FAB$^+$) calcd for $C_{24}H_{29}N_4O_2S$ ($M^+ + 1$) 437.2011, found 437.1968. Anal. ($C_{24}H_{28}N_4O_2S$) C, H, N.

4-(Benzylamino)-6-(benzylthio)-2-(dibutylamino)pyrimidine-5-carboxylic Acid (48). The title compound **48** (18.0 mg, 69%) was prepared from **18** (22.0 mg, 54.3 μmol) and phenyl methanethiol (13.5 mg, 0.109 mmol) in the same manner as described for **46**. Colorless solid. Mp: 138−139 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.70−0.85 (6H, m), 1.08−1.23 (4H, m), 1.30−1.55 (4H, m), 3.23−3.54 (14H, m), 4.27 (2H, s), 4.64 (2H, t, J = 4.9 Hz), 7.16−7.40 (10H, m), 8.85−9.00 (1H, br), 12.80−13.10 (1H, br). HRMS (FAB$^+$) calcd for $C_{27}H_{35}N_4O_2S$ ($M^+ + 1$) 479.2481, found 479.2491. Anal. ($C_{27}H_{34}N_4O_2S$ 0.1H_2O) C, H, N.

4-(Benzylamino)-2-(methylthio)-6-(2-thienyl)pyrimidine-5-carboxylic Acid (49). Step 1. Methyl 4-(benzylamino)-2-(methylthio)-6-(2-thienyl)pyrimidine-5-carboxylate. To a mixture of **13** (97.2 mg, 0.300 mmol) and 2-thienylboronic acid (57.6 mg, 0.450 mmol) in toluene (2 mL) and 1,4-dioxane (2 mL) were added 2 M Na_2CO_3 (2 mL) and $Pd(PPh_3)_4$ (70.0 mg, 60.6 μmol), and the mixture was stirred under reflux for 2 h. The reaction mixture was cooled to room temperature and diluted with ethyl acetate. It was then washed with 2 M Na_2CO_3 and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (hexane/AcOEt = 5:1) of the residue gave **49 Step 1** (61.0 mg, 55%) as a pale yellow solid. Mp: 114−116 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.52 (3H, s), 3.66 (3H, s), 4.75 (2H, d, J = 5.5 Hz), 7.06 (1H, dd, J = 3.7 and 1.2 Hz), 7.25−7.37 (6H, m), 7.46 (1H, dd, J = 4.8 and 1.2 Hz), 7.80 (1H, t, J = 5.5 Hz). HRMS (EI) calcd for $C_{18}H_{17}N_3O_2S_2$ (M^+) 371.0762, found 371.0760. Anal. ($C_{18}H_{17}N_3O_2S_2$) C, H, N.

Step 2. 4-(Benzylamino)-2-(methylthio)-6-(2-thienyl)pyrimidine-5-carboxylic acid (**49**). The title compound **49** (38.0 mg, 79%) was prepared from the compound of Step 1 (50.0 mg, 0.135 mmol) in the same manner as described for **9**. Colorless solid. Mp: 142−144 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.40 (3H, s), 4.64 (2H, d, J = 5.5 Hz), 7.15 (1H, dd, J = 5.5 and 4.3 Hz), 7.20−7.27 (1H, m), 7.32 (4H, d, J = 4.3 Hz), 7.48 (1H, dd, J = 3.7 and 1.2 Hz), 7.75 (1H, dd, J = 4.9 and 1.2 Hz), 8.08 (1H, t, J = 5.5 Hz), 13.55 (1H, s). HRMS (EI) calcd for $C_{17}H_{15}N_3O_2S_2$ (M^+) 357.0606, found 357.0634. Anal. ($C_{17}H_{15}N_3O_2S_2$ 0.3H_2O) C, H, N.

4,6-Bis(benzylthio)-2-(methylthio)pyrimidine-5-carboxylic Acid (50). To a solution of **10** (120 mg, 0.502 mmol) in DMF (1 mL) were added potassium carbonate (138 mg, 0.999 mmol) and phenyl methanethiol (124 mg, 1.00 mmol) at room temperature, and the mixture was stirred at room temperature for 4 h. To the resulting mixture was added 2 N HCl (1 mL) under ice cooling. The precipitate was formed and collected by filtration, and dried in vacuo. The title compound **50** (180 mg, 86%) was given as a colorless solid. Mp: 187−189 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.55 (3H, s), 4.38 (4H, s), 7.22−7.42 (10H, m), 14.00−14.25 (1H, br). HRMS (FAB$^+$) calcd for $C_{20}H_{19}N_2O_2S_3$ ($M^+ + 1$) 415.0609, found 415.0578. Anal. ($C_{20}H_{18}N_2O_2S_3$) C, H, N.

PPARγ Transactivation Assay. Chinese hamster ovary K1 (CHO-K1) cells were seeded at 5×10^4 cells/well in 24-well plates and cultured in Ham's F12 medium containing 10% fetal calf serum for 24 h at 37 °C. Cells were cotransfected for 2 h with 40 ng of GAL4/human PPAR LBD, 400 ng of pFR-Luc, and 5 ng of pRL-TK per well using lipofectamine (Gibco BRL, Grand Island, NY). Transfected cells were treated with various concentrations of test compounds at 37 °C for 20 h. Cells were washed with phosphate-buffered saline and dissolved in passive lysis buffer (Promega, Madison, WI). Luciferase activity was determined with a microplate luminescence reader, LUCY2 (Authors, Salzburg, Austria). The EC_{50} values of tested compounds were derived from the curve fitting using the Prism program (GraphPad Software, San Diego, CA). The standard reference full agonist is rosiglitazone (EC_{50} = 0.1 μM, 100% maximum activation at 10 μM). All results were produced in triplicate, and mean values are reported.

3T3-L1 Preadipocyte Differentiation Study. Confluent 3T3-L1 preadipocytes were treated with complete medium containing 1 μM dexamethasone, 1 μM IBMX, and 2 μM insulin (DEX/MIX medium). After 2 days, the DEX/MIX medium was removed, and cells were washed three times with medium and then treated with ligands at 37 °C for 48 h. The cells were stained by Oil-red O using adipogenesis assay kit (Hokudo).

In Vivo Study. Male *db*/*db* mice (7−8 weeks old) and lean mice were orally dosed once daily with test compounds and vehicle (0.25% methylcellulose) by oral gavage for 7 days. Mice (seven per group) received a once daily oral dosing of test compounds with vehicle (0.25% methylcellulose). Plasma glucose levels were measured before dosing on day 7. Each data point represents the mean value (±SD) of five individual mice.

Structure Determination. Crystal was soaked for 2 weeks in reservoir solution, which was prepared by the addition of 50 μL of compound solution (5.6 mg/1 mL EtOH) to 0.95 M sodium citrate and 100 mM HEPES buffer (pH 7.5). The crystals were cryoprotected in glycerol and flash frozen in a cryo-stream. X-ray data were collected using a Rikagaku RU-H2R X-ray generator and a Rikagaku RAXIS-V detector. The data were scaled and processed with the programs DPS-Mosflm and SCALA.[40,41]

The structure was determined by the molecular replacement method using the model structure (PDBID: 1PRG).[42] Refinement was carried out using the program CNX.[43] Model building and validation took place using the programs TOM-FLODO and PROCHECK.[44,45] A summary of the crystallographic statistics is shown in the Supporting Information.

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Supporting Information Available: Experimental details of compounds S1−S10; elemental analysis; summary of crystallographic statistics. This material is available free of charge via the Internet at http://pubs.acs.org.

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