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A new class of non-thiazolidinedione, non-carboxylic-acid-based highly selective peroxisome proliferator-activated receptor (PPAR) γ agonists: Design and synthesis of benzylpyrazole acylsulfonamides

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ABSTRACT

Herein, we describe the design, synthesis, and structure–activity relationships of novel benzylpyrazole acylsulfonamides as non-thiazolidinedione (TZD), non-carboxylic-acid-based peroxisome proliferator-activated receptor (PPAR) γ agonists. Docking model analysis of in-house weak agonist **2** bound to the reported PPARγ ligand binding domain suggested that modification of the carboxylic acid of **2** would help strengthen the interaction of **2** with the TZD pocket and afford non-carboxylic-acid-based agonists. In this study, we used an acylsulfonamide group as the ring-opening analog of TZD as an isosteric replacement of carboxylic acid moiety of **2**; further, preliminary modification of the terminal alkyl chain on the sulfonyl group gave the lead compound **3c**. Subsequent optimization of the resulting compound gave the potent agonists **25c**, **30b**, and **30c** with high metabolic stability and significant antidiabetic activity. Further, we have described the difference in binding mode of the carboxylic-acid-based agonist **1** and acylsulfonamide **3d**.

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterized by chronic hyperglycemia resulting from insulin resistance and impaired insulin secretion, leading to abnormal metabolism of not only glucose but also lipids and amino acids. Long-term microvascular and macrovascular complications such as neuropathy, retinopathy, nephropathy, myocardial infarction, stroke, and lower limb amputation, which develop as the disease progresses, gradually decrease quality of life of diabetic patients.[1] Although several drug treatments are currently available, the prevalence of T2DM is still increasing rapidly in both developed and developing countries, and the number of diabetics is expected to reach 380 million by 2025.[2] Therefore, there is an urgent need to develop new and safe antidiabetic agents capable of lowering hemoglobin A_{1c} (HbA_{1c}) levels while improving the lipid profile of patients.[3]

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. Three PPAR subtypes, namely, PPARα, PPARγ, and PPARδ (also known as PPARβ), have been cloned and characterized. Their effects are exerted through ligand-dependent transcription of a constellation of genes encoding proteins that regulate nutrient metabolism, energy homeostasis, and cell differentiation. PPARγ, the most extensively investigated subtype, is predominantly expressed in adipose tissue, and to a lesser extent, in the intestine, mammary gland, endothelium, liver, skeletal muscle, and in other tissues throughout the body, and plays a pivotal role in adipogenesis, glucose and lipid homeostasis, insulin sensitivity, inhibition of inflammatory responses, cell proliferation, and promotion of terminal differentiation.[4]

The introduction of thiazolidinediones (TZDs), represented by troglitazone (NOSCAL® or REZULIN®), pioglitazone hydrochloride

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(ACTOS®) and rosiglitazone maleate (AVANDIA®), as insulin sensitizers, and the discovery that TZDs are high-affinity PPARγ ligands,[5] has led to extensive research in the area of antidiabetic drug discovery and development.[6]

In the last two decades, efforts have been directed toward identifying novel classes of PPAR ligands as second-generation insulin sensitizers by using several approaches including PPARα/γ dual agonists, PPARγ/δ dual agonists, and PPARα/γ/δ pan agonists.[6a] Numerous non-TZD PPARγ ligands belonging to different chemical classes have been reported so far; however, most of these ligands are carboxylic acids. Thus, in this study, we investigated a new class of non-TZD and non-carboxylic-acid-based PPARγ agonists.

In this article, we report the design, synthesis, and biological evaluation of benzylpyrazole acylsulfonamides as a novel class of non-TZD, non-carboxylic-acid-based selective PPARγ agonists. In the optimization study, we identified potent, highly selective, and orally active PPARγ agonists, namely, **25c**, **30b**, and **30c**, which exhibited significant antidiabetic effects in diabetic rodent models. X-ray crystallographic analysis revealed that compound **3d** had a unique binding mode, especially of the acidic functionality, which was different from that observed for compound **1** in the docking model. Furthermore, we discuss the key elements of PPARγ selectivity of these compounds.

2. Lead generation

Several X-ray co-crystal structures of PPARγ ligand-binding domain (LBD) in complex with various ligands have been solved and the protein-ligand interactions have been clarified at atomic resolution. These studies suggest that the PPARγ-LBD comprises 13 α-helices and a small 4-stranded β-sheet. The helices 3, 7, and 10 form the Y-shaped ligand-binding site that is located in the bottom half of the overall structure.[7] The X-ray co-crystal analysis of rosiglitazone with PPARγ-LBD indicated that the former binds to the LBD in a U-shaped motif and the TZD head group interacts with Tyr473 located in activation function 2 (AF-2) helix. In the co-crystal structure of rosiglitazone, the tail pyridine ring occupies 1 pocket of the Y-shaped cavity comprising hydrophobic amino acids such as Ile281, Met348, Ile341 but it does not use the second pocket comprising amino acids such as Phe226, Leu228, Met329.[7a]

We previously identified benzylpyrazole phenylacetic acid **1** as a potent PPARγ agonist with a half-maximal effective concentration (EC_{50}) value of 2.3 nM.[8] However, its synthetic precursor, (1-benzylpyrazol-5-yl)propanoic acid derivative **2** proved to be a weak PPARγ agonist with an EC_{50} value of 500 nM (Fig. 1). We obtained weak or virtually inactive compounds with our initial efforts to identify non-carboxylic-acid-based agonists using compound **1**-related phenylacetic acids (data not shown). On the basis of these results, we speculated that the difference in the transactivation activities resulted from how the compounds occupied all the 3 arms of the Y-shaped cavity. To confirm our assumption, we performed a docking study of compounds **1** and **2**. Previously reported PPARγ-LBD structures in complex with rosiglitazone (PDB code: 1FM6)[9] were used for the docking of compounds **1** and **2**. The compounds were docked into the PPARγ-LBD using the GOLD program (ver. 2.0, the Cambridge Crystallographic Data Centre, UK). Docking positions with a high score were corrected manually to avoid small steric bumps, minimized energetically using MMFF94s force field in MOE program (Molecular Operating Environment, Chemical Computing Group Inc., Canada), and plausible binding modes were obtained.

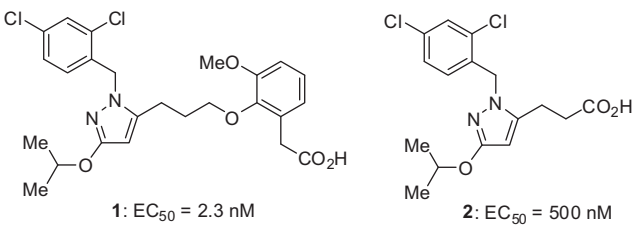


Figure 1. Chemical structures and in vitro PPARγ transactivation activities of benzylpyrazole-based PPARγ agonists **1** and **2**.

The docking models of **1** and **2** superposed with the co-crystal structure with rosiglitazone (PDB code: 1FM6) are shown in Figure 2. Rosiglitazone binds to the Y-shaped cavity of the PPARγ-LBD in a U-shaped motif and the TZD head group interacts with Tyr473 of the AF-2 helix. Conversely, as expected, the docking study indicates that compound **1** fully occupies all three arms of the Y-shaped cavity including the 'ligand entrance' pocket that is not occupied by rosiglitazone. The phenylacetic acid moiety of **1** effectively occupies 'the TZD pocket' (which accommodates the TZD group of rosiglitazone); further, similar to the TZD group of rosiglitazone, the carboxyl group forms hydrogen bonds with Tyr473 as well as Ser289 and His323. This model suggests that there is little space for accommodating an additional group in the TZD pocket, which is consistent with the fact that further modification of the carboxylic acid of **1** remarkably decreases the potency.

In the docking model of compound **2**, the pyrazole ring slightly shifts to the right as compared to that in **1**; nevertheless, the binding position of the benzylpyrazole moiety of **2** is very similar to that of **1**. The carboxylic acid of **2** does not fully occupy the TZD pocket or form a hydrogen bond with Tyr473, which is consistent with weak agonism. The binding mode of **2** implies that the benzylpyrazole substructure, not the acidic group, serves as the anchor since the former occupies the two binding pockets of the Y-shaped cavity through strong hydrophobic interactions. Based on this observation, we assumed that modification of the carboxylic acid of **2** could strengthen the interaction with the TZD pocket and afford potent non-carboxylic-acid-based agonists.

Therefore, we opted to pursue the benzylpyrazole chemotype starting with compound **2** to identify a new class of non-TZD and non-carboxylic-acid-based PPARγ agonists.

To date, heterocyclic moieties such as TZDs, oxazolidinediones, and tetrazoles have been widely incorporated into PPAR ligands as isosteric replacements of the carboxylic acid.[6] In contrast, acyclic isosteres of carboxylic acids have drawn little attention and have

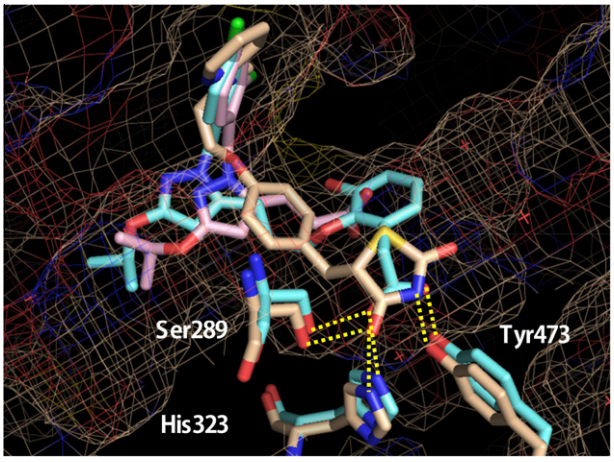


Figure 2. Docking models of the phenylacetic acid **1** (shown in cyan) and the propionic acid **2** (shown in purple) bound to the co-crystal structure of PPARγ-LBD [PDB code: 1FM6] with rosiglitazone (shown in peach). Nitrogen, oxygen, sulfur, and chlorine atoms are colored in blue, red, yellow, and light green, respectively. Ser289, His323, and Tyr473 interacting with the TZD of rosiglitazone or the carboxylic acid of **1** are highlighted.

been rarely reported.[10] In this study, we focused on an acylsulfonamide group as a ring-opened analog of TZD.[11] Similar to the interactions with rosiglitazone, we expected that one or two oxygen atoms of a flexible acylsulfonamide group might interact with the key amino acid residues in the TZD pocket (Fig. 2). Furthermore, preparation of acylsulfonamide from the corresponding carboxylic acid is easy and the terminal substituent is expected to contribute to increase in binding energy. Indeed, preliminary introduction of alkylsulfonamides into **2** gave encouraging results. While the potency of methylsulfonamide **3a** was comparable to that of the parent carboxylic acid **2**, the potency of propylsulfonamide **3b** and pentyl analog **3c** was about threefold and 30-fold more potent, respectively. Subsequently, we conducted optimization studies of the benzylpyrazole acylsulfonamides using **3c** as the starting compound.

3. Chemistry

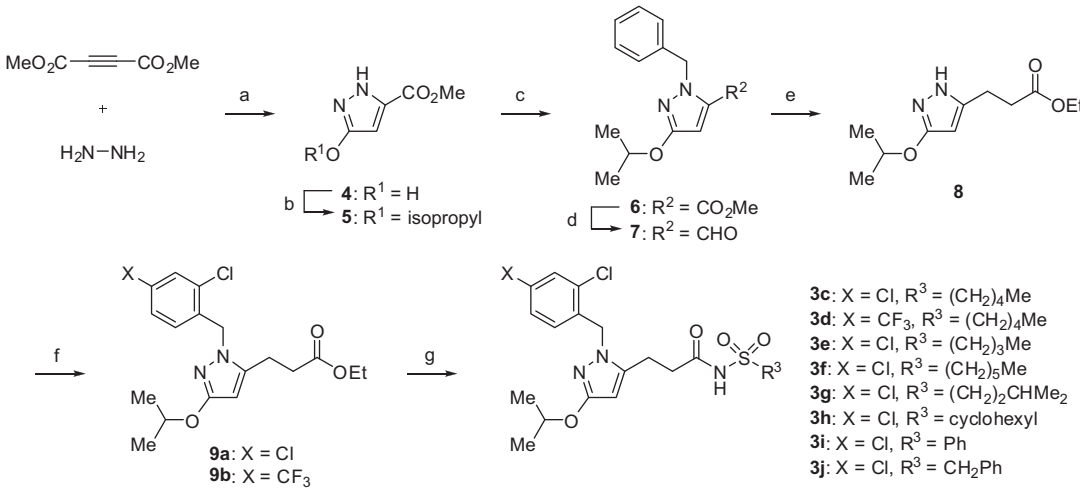
Benzylpyrazole acylsulfonamides are generally synthesized by the route shown in Scheme 1. Cyclization of dimethyl acetylenedicarboxylate (DMAD) with hydrazine proceeded smoothly to afford hydroxypyrazole **4**, which was alkylated successively with 2-iodopropane and benzyl bromide to produce ester **6**. Both alkylations occurred at the desired positions with moderate regioselectivity, and regioisomers of **6** were easily separated by silica gel chromatography. The chemical structure of **6** was determined by two-dimensional nuclear magnetic resonance (2D NMR) spectroscopy (data not shown). Reduction of ester **6** with diisobutylaluminum hydride (DIBAL-H) followed by Swern oxidation gave aldehyde **7**. Horner–Wadsworth–Emmons reaction with compound **7** and triethyl phosphonoacetate afforded the corresponding α,β-unsaturated ester. Subsequent catalytic hydrogenation resulted in removal of the benzyl group and concomitant saturation of the double bond to afford ester **8**. N-benzylation of **8** and subsequent saponification yielded carboxylic acids, which were condensed with sulfonamides to produce the corresponding acylsulfonamides 3c–**j**. The regioselectivity of the benzylation of **8** was moderate similarly to that of **6**, and the regioisomers of the benzylated products were also separated by silica gel chromatography.

The synthesis of the 1-benzylpyrazoles with different 3-alkoxy groups was performed according to Scheme 2. Reaction of methyl carbazate with 2,4-dichlorobenzyl chloride (**10**) gave *N*,*N*′-disubstituted hydrazine derivative **11**. Michael addition of **11** to DMAD took place at the nitrogen adjacent to the benzyl group with high regioselectivity. The resulting adduct was treated with sodium methoxide with heating, which resulted in the removal of the *N*-methoxycarbonyl group and subsequent cyclization afforded 3-hydroxypyrazole **12**. The 3-hydroxy group was protected by a methoxymethyl (MOM) group, and the resulting ester was converted to α,β-unsaturated ester **14** by following steps similar to those described in Scheme 1. Catalytic hydrogenation of **14** and subsequent treatment with hydrochloric acid gave 3-hydroxypyrazole **16**, which was O-alkylated to provide 3-alkoxypyrazoles **17a–d**. Alkaline hydrolysis and subsequent condensation with 1-pentanesulfonamide afforded the acylsulfonamides **18a–d**.

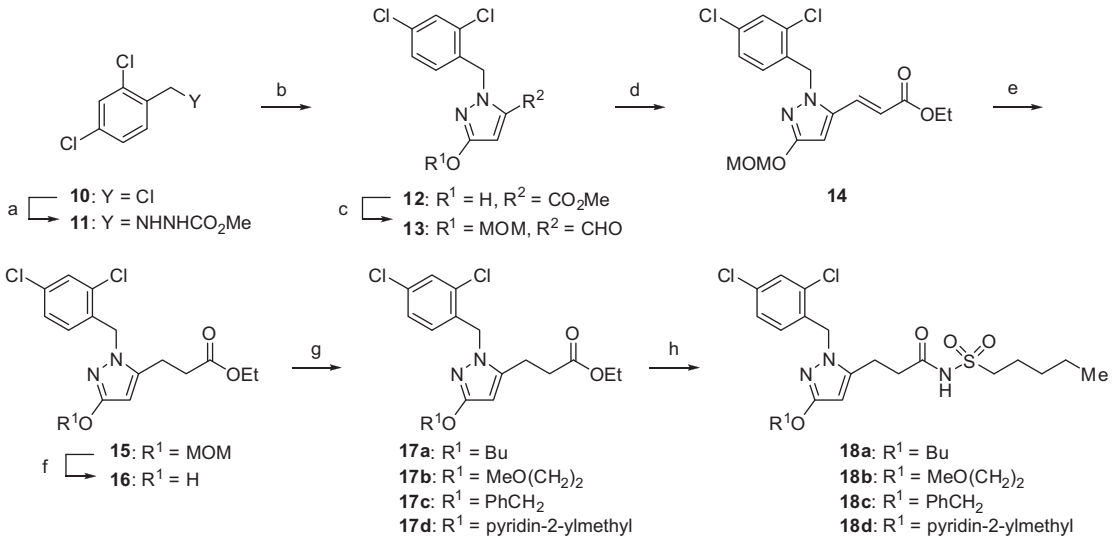
The synthetic scheme of 3-alkylpyrazole acylsulfonamides is shown in Scheme 3. Claisen condensation of ketones **19a–c** with diethyl oxalate afforded the corresponding α,γ-diketoesters, which were reacted with hydrazine to obtain the corresponding 3-alkylpyrazoles **20a–c**. The following sequence of reactions consisting of the introduction of 1-benzyl group, reduction of the ester group, Swern oxidation, Horner–Wadsworth–Emmons reaction, reduction of the spacer double bond, and construction of acylsulfonamide were performed according to the standard procedure as mentioned above to give acylsulfonamides **24a–d**. Finally, intermediary esters **22a**,**c** were hydrolyzed and subsequently coupled with 1-pentanesulfonamide to provide the corresponding propenoylsulfonamides **25a**,**c**.

Benzylpyrazole propenoylsulfonamides with a 3-alkoxy group on the pyrazole ring were prepared as shown in Scheme 4. Pyrazole **4** was successively alkylated with MOM chloride and benzyl chloride to provide ester **26** according to the standard procedure. Aldehyde **27**, which was prepared from **26** via a 3-step protocol, that is, hydrolysis, Weinreb amide formation, and reduction with DIBAL-H, was subjected to Horner–Wadsworth–Emmons reaction to yield α,β-unsaturated ester **28**. The analogous ester **14** prepared in Scheme 2 and compound **28** were used for further transformation, wherein **14** and **28** were converted to the acylsulfonamides **30a–d** by a procedure similar to that described in Scheme 2.

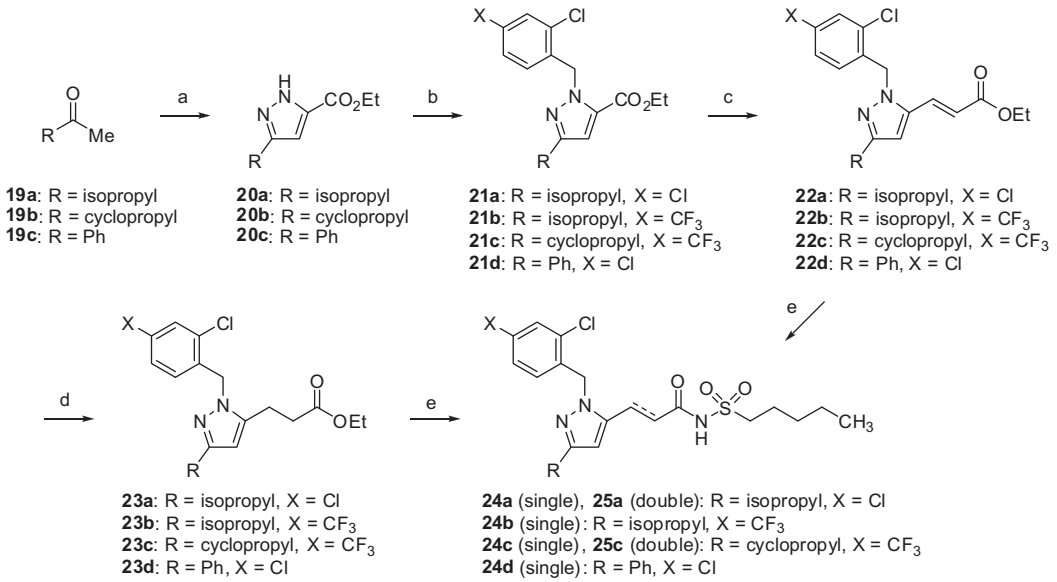
The spacer length between the pyrazole ring and acylsulfonamide group was modified according to Scheme 5. The synthesis was initiated by O-butylation of 3-hydroxypyrazole **12** to give ester **31**, which was hydrolyzed to produce carboxylic acid **33a**. Ester **31** was also reduced with DIBAL-H and the resulting alcohol was reacted with acetone cyanohydrin under Mitsunobu conditions to give cyanide **32a**,[12] which was converted to acetic acid **33b** by saponification. Similarly, butanoic acid **33c** was obtained from



Scheme 1. Synthesis of **3c–j**. Reagents and conditions: (a) AcOH, toluene, rt; (b) 2-iodopropane, K_2CO_3, DMF, rt; (c) $PhCH_2Br$, K_2CO_3, DMF, rt; (d) (i) DIBAL-H, THF, rt; (ii) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, then Et_3N, rt; (e) (i) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, DMF, 0 °C–rt; (ii) HCO_2H, Pd/C, EtOH, reflux; (f) 2,4-dichlorobenzyl chloride, NaH, DMF, rt for **9a**; 2-chloro-4-trifluoromethylbenzyl chloride, NaH, DMF, rt for **9b**; (g) (i) 1 M NaOH, THF, EtOH, 50 °C; (ii) CDI, THF, reflux, then RSO_2NH_2, DBU, rt.



Scheme 2. Synthesis of **18a–d**. Reagents and conditions: (a) methyl carbazate, K_2CO_3, DMF, 80 °C; (b) $MeO_2CC{\equiv}CCO_2Me$, MeOH, reflux, then NaOMe, reflux; (c) (i) MOMCl, K_2CO_3, THF, DMF, rt; (ii) DIBAL-H, THF, 0 °C–rt; (iii) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, then Et_3N, rt; (d) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, DMF, 0 °C–rt; (e) H_2, Pd/C, THF, rt; (f) HCl, EtOH, reflux; (g) ROH, Bu_3P, ADDP, THF, rt for **17a–c**; 2-(chloromethyl)pyridine hydrochloride, K_2CO_3, DMF, rt for **17d**; (h) (i) 1 M NaOH, THF, EtOH, 50 °C; (ii) CDI, THF, reflux, then 1-pentanesulfonamide, DBU, rt for **18a–c**; CDI, DMF, rt, then 1-pentanesulfonamide, DBU, 100 °C for **18d**.



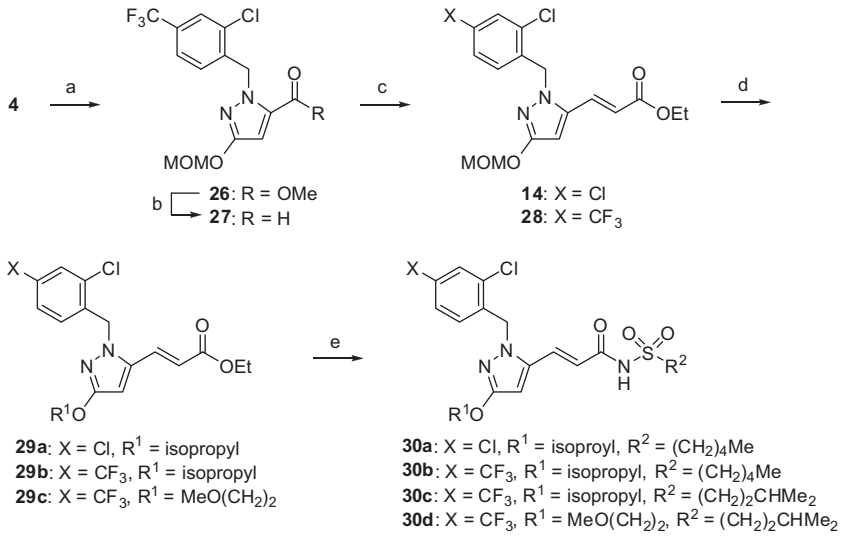
Scheme 3. Synthesis of **24a–d** and **25a,c**. Reagents and conditions: (a) (i) $(CO_2Et)_2$, *t*-BuOK, THF, rt; (ii) $H_2NNH_2{\cdot}H_2O$, AcOH, THF, reflux; (b) 2,4-dichlorobenzyl chloride, K_2CO_3, DMF, rt for **21a,d**; 2-chloro-4-trifluoromethylbenzyl chloride, K_2CO_3, DMF, rt for **21b,c**; (c) (i) DIBAL-H, THF, rt; (ii) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, then Et_3N, rt; (iii) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, DMF, rt; (d) H_2, Pd/C, THF, rt; (e) (i) 1 M NaOH, THF, EtOH, 50 °C; (ii) CDI, THF, reflux, then 1-pentanesulfonamide, DBU, rt for **24a,b,d**, **25a**; CDI, DMF, rt, then 1-pentanesulfonamide, DBU, 100 °C for **24c**,**25c**.

propionic acid ester **17a** via cyanide **32b**. Furthermore, butanoic acid **33c** was homologated in a similar manner as that described for **32a**,**b** to afford pentanoic acid **33d**. Condensation of the carboxylic acids **33a–d** with 1-pentanesulfonamide afforded the acylsulfonamides **34a–d**.

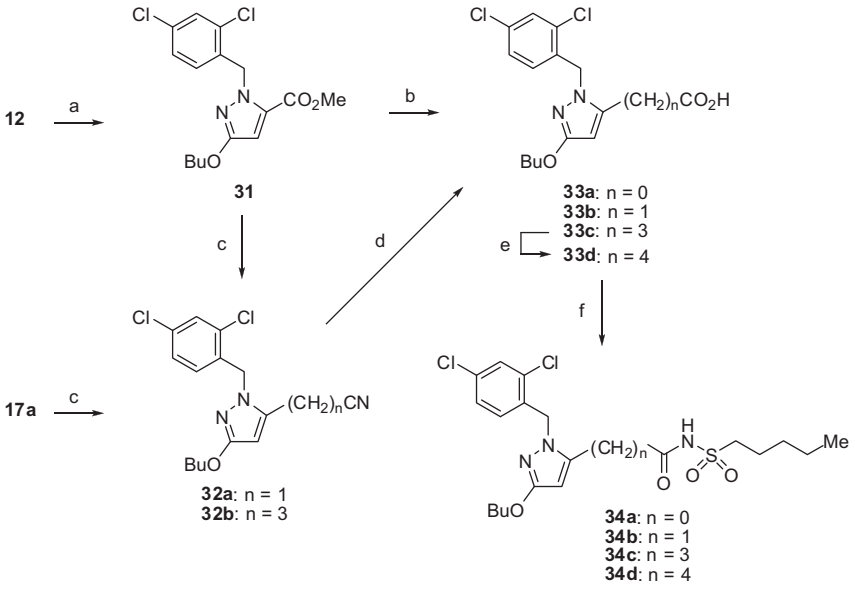
4. Results and discussion

4.1. Human PPARs transactivation activity and metabolic stability

We evaluated the transactivation activity of the newly synthesized benzylpyrazole-based PPARγ ligands against human PPARγ stably expressed in Chinese hamster ovary (CHO) cells and their metabolic stability in human liver microsomes. We also evaluated the transactivation activities of these compounds against the other 2 PPAR subtypes, namely, human PPARα and PPARδ, which were transiently expressed in COS-1 cells. Transactivation activity was assessed by a luciferase reporter gene assay and the activities were reported as EC_{50} value. (*R*)-5-(3-{4-[(2-Furan-2-yl-5-methyl-1,3-oxazol-4-yl)methoxy]-3-methoxyphenyl}propyl)-1,3-oxazolidine-2,4-dione,[13] ({3-[(5-methyl-2-phenyl-1,3-oxazol-4-yl)methoxy]benzyl}sulfanyl) acetic acid,[14] and carbacyclin[15] were used as the reference PPARγ, α, and δ agonists, respectively. Metabolic stability was assessed by measuring clearance rate of the parent compound after treatment with human liver microsomes (see Section 6).



Scheme 4. Synthesis of **30a**–**d**. Reagents and conditions: (a) (i) MOMCl, K_2CO_3, DMF, 0 °C–rt; (ii) 2-chloro-4-trifluoromethylbenzyl chloride, K_2CO_3, DMF, rt; (b) (i) 1 M NaOH, THF, MeOH, 50 °C; (ii) MeONHMe·HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI), HOBt, DMF, rt; (iii) DIBAL-H, THF, 0 °C; (c) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, DMF, 0 °C–rt; (d) (i) concd. HCl, EtOH, reflux; (ii) 2-iodopropane, K_2CO_3, DMF, rt for **29a**,**b**; 2-methoxyethanol, Bu_3P, ADDP, THF, rt for **29c**; (e) (i) 1 M NaOH, THF, EtOH, 50 °C; (ii) CDI, DMF, rt, then 1-pentanesulfonamide, DBU, 100 °C for **30a**,**b**; CDI, DMF, rt, then 3-methylbutane-1-sulfonamide, DBU, 100 °C for **30c**,**d**.



Scheme 5. Synthesis of **34a**–**d**. Reagents and conditions: (a) BuOH, PBu_3, ADDP, THF, rt; (b) 1 M NaOH, THF, EtOH, 50 °C; (c) (i) DIBAL-H, THF, 0 °C; (ii) acetone cyanohydrin, Bu_3P, ADDP, THF, rt; (d) 4 M NaOH, EtOH, reflux; (e) (i) BH_3·THF, THF, rt; (ii) acetone cyanohydrin, Bu_3P, ADDP, THF, rt; (iii) 4 M NaOH, EtOH, reflux; (f) CDI, THF, reflux, then 1-pentanesulfonamide, DBU, rt.

Despite showing potent PPARγ agonism, by oral administration the lead compound **3c** exhibited weak glucose-lowering effects in diabetic rodent models. The preliminary pharmacokinetic data of **3c** were obtained by cassette dosing in rats. After oral administration (1 mg/kg), compound **3c** showed insufficient pharmacokinetic profile (CL_{total} = 794 mL/h/kg, $AUC_{0-8\ h}$ = 495.3 ng h/mL, F = 37.6%), presumably due to poor metabolic stability in hepatic microsomes (metabolic clearance h/r = 195/128 μL/min/mg). Additionally, incubation of **3c** with hepatic microsomes indicated that the isopropoxy group and the pentyl side chain were metabolically labile in both humans and rats, which supported the aforementioned result (data not shown). Thus, we aimed to improve metabolic stability and study the structure–activity relationship (SAR) of the chemotype. Our strategy to improve metabolic stability was based on reduction of molecular lipophilicity, increment in molecular rigidity, and/or protection of the metabolically labile sites.

As shown in Figure 3, alkyl substituents of sulfonamide group exhibited positive effects on PPARγ activity. Thus, we studied the SAR by further examining the terminal side chain on the sulfonyl group with 1-(2,4-dichlorobenzyl)-3-isopropoxypyrazole derivative **3c**; the results are presented in Table 1. Both shortening (**3e**) and elongation (**3f**) of the alkyl chain decreased the activity, while branching at the terminal of the alkyl chain (**3g**) maintained the activity. Incorporation of large substituents such as cyclohexyl (**3h**), phenyl (**3i**), or benzyl (**3j**) groups resulted in more than six-fold drop in potency. Nevertheless, all these compounds, except **3j**, served as selective PPARγ agonists (EC_{50} >10000 nM for PPARα and PPARδ) similar to **3c**. With regard to metabolic stability, no

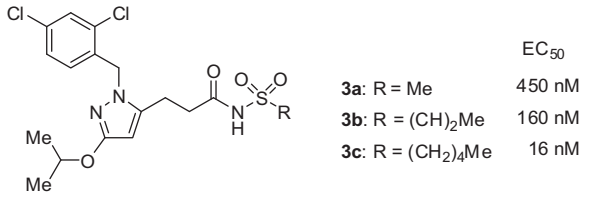
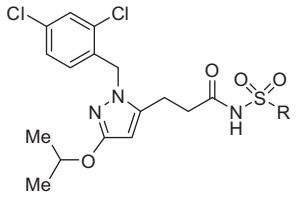


Figure 3. In vitro PPARγ transactivation activities of benzylpyrazole-based PPARγ agonists **3a–c**.

Table 1
In vitro PPARγ transactivation activities and metabolic clearance of compounds **3c**,**e–j**



Compound	R	PPARγ transactivation EC_{50} (nM)[a]	Metabolic clearance (μL/min/mg)[b]
3e	Butyl	40	165
3c	Pentyl	16	192
3f	Hexyl	21	—[c]
3g	Isoamyl	17	—[c]
3h	Cyclohexyl	150	—[c]
3i	Phenyl	110	—[c]
3j	Benzyl	260	107

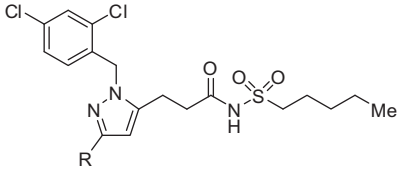
[a] EC_{50} value means the effective concentration for 50% response of a given compound's intrinsic maximum response.
[b] In vitro metabolic clearance was determined based on the disappearance of the parent compound after incubation with human liver microsomes for 20 min.
[c] Not determined.

improvement was observed after this modification consistent with their high lipophilicity. The result suggests that pentyl and isoamyl groups are optimal for potent PPARγ agonism and spatial limitation may exist for the binding pocket that accommodates this part of the molecule. Consequently, pentyl and isoamyl groups were selected as the tail alkyl chains for further SAR study.

Next, we examined the effects of the 3-substituent on the pyrazole ring on PPARs transactivation activity; the results are summarized in Table 2. Overall, a variety of lipophilic substituents like butoxy (**18a**) benzyloxy (**18c**), isopropyl (**24a**) and phenyl (**24d**) groups were tolerated at this position. These results are consistent with the docking study that the binding pocket interacting with this part of the ligand is relatively large and lipophilic. In addition, the methoxyethoxy group (**18b**) was also acceptable; however, improvement in the metabolic stability was minimal with these structural changes. Benzyloxy group (**18c**) retained the activity as compared with **3c**, whereas substitution of a pyridine ring (**18d**) for the benzene ring of **18c** decreased the activity. As for PPARα and δ transactivation activities, all compounds exhibited EC_{50} values of >10000 nM. Among these compounds, compound **18a** exhibited slightly higher PPARα activity (data not shown). According to the SAR results, isopropoxy, methoxyethoxy, isopropyl, and their related groups were investigated as the 3-substituent of the pyrazole in the subsequent optimization study (see Table 4).

The lead compound **3c** possesses unique molecular architecture, a lipophilic benzylpyrazole template linked by an ethylene spacer to an acylsulfonamide bearing a tail pentyl group. Unlike most of the non-carboxylic-acid-based PPARγ agonists, the acidic acylsulfonamide moiety of **3c** is located in the center of the molecule.

Table 2
In vitro PPARγ transactivation activities and metabolic clearance of compounds **3c**, **18a–d**, and **24a**,**d**



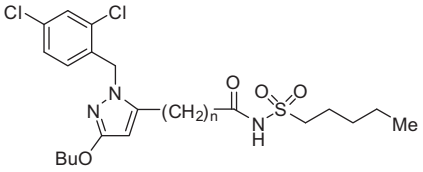
Compound	R	PPARγ transactivation EC_{50} (nM)[a]	Metabolic clearance (μL/min/mg)[b]
3c	Isopropoxy	16	192
18a	Butoxy	7.5	—[c]
18b	$MeO(CH_2)_2O$	31	144
18c	Benzyloxy	14	100
18d[d]	2-Pyridylmethoxy	120	224
24a	Isopropyl	13	124
24d	Phenyl	11	111

[a] EC_{50} value means the effective concentration for 50% response of a given compound's intrinsic maximum response.
[b] In vitro metabolic clearance was determined based on the disappearance of the parent compound after incubation with human liver microsomes for 20 min.
[c] Not determined.
[d] Hydrochloric acid salt.

Considering the difference in the activities of compounds **1** and **2**, the distance of the acidic function from the lipophilic template could affect PPARγ potency. This assumption prompted us to next investigate effects of the alkylene spacer length on agonism with 1-benzyl-3-butoxypyrazole analog **18a**. Shortening of the ethylene spacer of **18a** (**34a**,**b**) proved to be detrimental to PPARγ transactivation activity. Similarly, elongation of the spacer (**34c**,**d**) reduced the activity, but the agonists still retained 10^{-8} M potency (Table 3). Accordingly, we pursued optimization of this series of compounds with a 2-carbon spacer.

In medicinal chemistry, empirical observation suggests that less flexible molecules tend to have improved metabolic stability despite high lipophilicity.[16] Therefore, we next examined the effect of incorporation of a double bond into the spacer and replacement of the para substituent of the benzyl group and 3-substituent on the pyrazole ring; the results are presented in Table 4. Replacement of the chlorine atoms of **3c** and **24a** with a trifluoromethyl group (**3d**, **24b**) gave essentially equipotent activity. Interestingly, substitution of a cyclopropyl group (**24c**) for the isopropyl group of **24b** retained 10^{-8} M order potency with moderately improved metabolic stability. It is noteworthy that remarkable improvement in metabolic stability was generally achieved through the

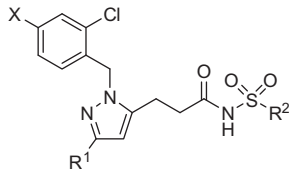
Table 3
In vitro PPARγ transactivation activities of compounds **18a** and **34a–d**



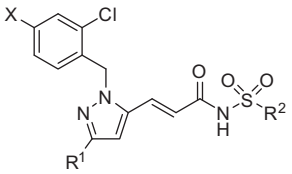
Compound	*n*	PPARγ transactivation EC_{50} (nM)[a]
34a	0	Inactive
34b	1	>10000
18a	2	7.5
34c	3	67
34d	4	29

[a] EC_{50} value means the effective concentration for 50% response of a given compound's intrinsic maximum response.

Table 4
In vitro PPARγ transactivation activities and metabolic clearance of compounds **3c**,**d**, **24a–c**, **25a**,**c**, and **30a–d**



3c, **3d**, **24a-c**



25a,**c**, **30a-d**

Compound	X	R^1	R^2	PPARγ transactivation EC_{50} (nM)[a]	Metabolic clearance (μL/min/mg)[b]
3c	Cl	Isopropoxy	Pentyl	16	192
24a	Cl	Isopropyl	Pentyl	13	124
3d	CF_3	Isopropoxy	Pentyl	10	163
24b	CF_3	Isopropyl	Pentyl	8.4	136
24c	CF_3	Cyclopropyl	Pentyl	13	78
30a	Cl	Isopropoxy	Pentyl	20	29
30b	CF_3	Isopropoxy	Pentyl	9.3	26
30c	CF_3	Isopropoxy	Isoamyl	8.3	32
30d	CF_3	$MeO(CH_2)_2O$	Isoamyl	9.0	34
25a	Cl	Isopropyl	Pentyl	8.2	99
25c	CF_3	Cyclopropyl	Pentyl	12	54

[a] EC_{50} value means the effective concentration for 50% response of a given compound's intrinsic maximum response.
[b] In vitro metabolic clearance was determined based on the disappearance of the parent compound after incubation with human liver microsomes for 20 min.

incorporation of a double bond into the spacer. For example, propenoylsulfonamide **30a** showed 6.6-fold lower metabolic clearance than the corresponding propanoylsulfonamide **3c** while maintaining the selective PPARγ activity. As in the case of compounds with the ethylene spacer (**3c**, **24a** vs **3d**, **24b**), replacement of the chlorine atom of **30a** with a trifluoromethyl group (**30b**,**c**) induced marginal increase in the potency. In contrast to the potency of the propanoylsulfonamide series compounds (**3c** vs **18b** shown in Table 2), the high potency was preserved by substitution of a methoxyethoxy group (**30d**) for the isopropoxy group of compound **30c**. Moreover, 3-alkylpyrazole series such as isopropyl (**25a**) and cyclopropyl (**25c**) derivatives showed about 10 nM potency with comparatively limited improvement in metabolic stability. Overall, the compounds **30b**, **30c**, and **30d** exhibited favorable metabolic stability and potent PPARγ agonism with EC_{50} values of 9.3, 8.3, and 9.0 nM, respectively. In addition, compound **25c** showed acceptable metabolic stability and potent activity with EC_{50} value of 12 nM. Greater than 500-fold subtype selectivity was observed as all compounds showed PPARα and δ transactivation activities with EC_{50} values of >10000 nM; however, 2-chloro-4-trifluoromethylbenzyl derivative **30c** and **30d** with isoamyl side chain exhibited a slightly high PPARα maximum response (48% and 47% of control at 10 μM, respectively). These results are consistent with the fact that the metabolic stability of this class of compounds depends on their flexibility rather than their lipophilicity.

In the view of the results of the SAR study and the structural diversity, we selected **3d**, **25c**, **30b**, and **30c** as potent and highly selective PPARγ agonists for further in vitro and in vivo evaluations.

4.2. PPARγ binding activity

To confirm that the representative agonists **3d**, **25c**, **30b**, and **30c** activate PPARγ through direct binding to the receptor, we performed a competition binding assay using GST-hPPARγ and the radioligand [^{3}H]-AD-5061 according to a modified protocol of our previously established method.[17a] With regard to the PPAR subtype selectivity of the above mentioned compounds, they did not exhibit significant activities against PPARα and PPARδ at 10 μM, indicating that they are highly selective PPARγ ligands. As shown in Table 5, these agonists robustly inhibited the specific binding of [^{3}H]-AD-5061 to human PPARγ with IC_{50} values ranging from 12 to 23 nM, which were similar to the EC_{50} values of their functional activities. The results suggest that the transactivation activity of the compounds is attributed to direct binding to the receptor, implying that the affinity of these compounds can be increased by occupying all 3 arms of the Y-shaped cavity of the PPARγ-LBD. Indeed, the effective occupancy of the Y-shaped cavity by these agonists was confirmed in an X-ray co-crystallographic study to be discussed later. These results clearly show that the agonists effectively bind to the PPARγ-LBD and result in a potent activation of PPARγ.

4.3. In vivo antidiabetic effects in rodent models of T2DM

We next evaluated metabolic stability and pharmacokinetic profile in rats as well as the in vivo antidiabetic efficacy of the benzylpyrazole acylsulfonamides in a genetically obese rodent model of T2DM, fed male Wistar fatty rats.[17b] The selected compounds **25c**, **30b**, and **30c** with high metabolic stability were orally administered to male Wistar fatty rats for 7 days. As shown in Table 6, these agonists significantly reduced plasma glucose (PG) levels with ED_{25} values ranging from 0.12 to 0.42 mg/kg and triglyceride (TG) levels with ED_{25} values ranging from 0.04 to 0.55 mg/kg in a

Table 5
PPARs transactivation activities and PPARγ binding affinities of compounds **3d**, **25c**, **30b**, **30c**

Compound	PPARs transactivation EC_{50} (nM)[a]			PPARγ binding
	PPARγ	PPARα	PPARδ	IC_{50} (nM)[b]
3d	10	>10000	>10000	21
25c	12	>10000	>10000	12
30b	9.3	>10000	>10000	23
30c	8.3	>10000	>10000	15

[a] EC_{50} value means the effective concentration for 50% response of a given compound's intrinsic maximum response.
[b] IC_{50} value means the concentration of a given compound required for 50% inhibition of the specific binding of [^{3}H]-AD-5061 to human PPARγ.

Table 6
PPARγ transactivation activities, plasma glucose (PG)- and triglyceride (TG)-lowering effects in Wistar fatty rats, and metabolic clearance by treatment with rat microsomes of compounds **25c**, **30b**, and **30c**

Compound	PPARγ transactivation EC_{50} (nM)[a]	Wistar fatty rats [b]		Rat metabolic clearance (μL/min/mg)[e]	$AUC_{0\text{-}8h}$ (ng h/mL)[f]
		PG ED_{25} (mg/kg)[c]	TG ED_{25} (mg/kg)[d]		
25c	12	0.42	0.55	116	325.6
30b	9.3	0.12	0.04	33	2487.7
30c	8.3	0.21	0.09	51	2888.1

[a] EC_{50} value means the effective concentration for 50% response of a given compound's intrinsic maximum response.
[b] Test compounds were administered by oral gavage to male Wistar fatty rats for 7 days.
[c,d] Effective dose for 25% reduction of plasma glucose (PG) and triglyceride (TG), respectively.
[e] EC_{50} value means the effective concentration for 50% response of a given compound's intrinsic maximum response. Rat liver microsome was used instead of human liver microsome.
[f] Rat cassette dosing at 1 m/kg, po. Average of $AUC_{0-8\ h}$ values in three rats.

dose-dependent manner. The improved metabolic stability of **30b** and **30c** is considered to be reflected in the higher plasma exposure ($AUC_{0-8\ h}$). Furthermore, a good correlation between the in vivo potency of the compounds in Wistar fatty rats and their plasma exposure in rats was observed, indicating that potent antidiabetic efficacy of **30b**, **30c** is mainly attributed to not only potent transactivation activity but also improved pharmacokinetic profile. These results clearly demonstrated that these agonists exhibited significant antidiabetic effects.

4.4. Docking studies based on X-ray co-crystallographic analyses of PPAR ligands

To gain a structural insight into the molecular basis underlying the interaction of acylsulfonamide-based PPARγ agonists, we analyzed the co-crystal structure of human PPARγ-LBD in complex with the compound **3d**. The binding mode of **3d** to the PPARγ-LBD obtained by the co-crystallographic analysis is shown in Figure 4A. The binding position of the benzylpyrazole moiety of **3d** was found to be similar to that observed in the docking model of propionic acid **2** to the co-crystal structure of the PPARγ-LBD [PDB code: 1FM6] (see Fig. 2). The 2-nitrogen atom of the pyrazole ring interacted with the backbone amide oxygen of Leu340 through a water molecule-mediated hydrogen bond. Both the hydrophobic and hydrogen-bonding interactions contribute to effective occupancy of the two arms of the Y-shaped cavity. The pentylsulfonylamino group adopts two conformations, which are similar except for their terminal pentyl group. In one conformation, the acylsulfonamide moiety forms the following 3 hydrogen bonds: with Tyr327 via the carbonyl oxygen, with His449 via one sulfonyl oxygen, and with Gln286 via the other sulfonyl oxygen. In the other conformation, it forms two hydrogen bonds with Tyr327 and His 449.

The result of superposition with the co-crystal structure of the acylsulfonamide **3d** and docking model of the phenylacetic acid **1** to the PPARγ-LBD is shown in Figure 4B. The main difference between the two binding modes is the interaction of the acidic groups with the key amino acid residues in the TZD pocket. The carboxyl group of **1** directly interacts with Tyr473 through hydrogen bonding. However, the acylsulfonamide moiety of **3d** does not interact with Tyr473, but it interacts with Tyr327, His449, and/or Gln286, as mentioned above. The other difference between the two LBD structures is the conformation of the phenyl side chain of Phe363 located in helix 3. The phenyl side chain in the docking model of **1** covers the TZD pocket from above, whereas the side chain of Phe363 in the co-crystal structure of **3d** adopts a 'flipped-out' conformation, opening up an additional cleft-like lipophilic binding space that vertically extends from the middle of the TZD pocket. Thus, Phe363 is considered as a 'gate-keeper' residue since it is located in the entrance region of the newly generated cleft[18]; the additional binding region can accommodate the pentylsulfonyl group. We speculate that the acylsulfonamide derivatives that appropriately occupy all three arms of the Y-shaped cavity as well as the new binding cleft adjacent to the TZD pocket by flipping the 'gate-keeper' Phe363 residue could compensate for decreased binding energy derived from no interaction with Tyr473, leading to potent PPARγ agonism.

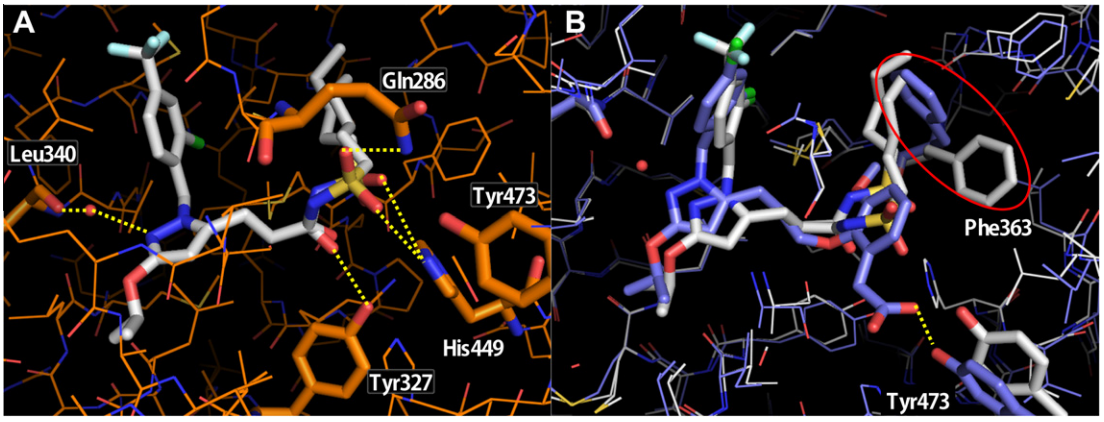


Figure 4. (A) Co-crystal structure of the acylsulfonamide-based agonist **3d** (shown in gray) with PPARγ-LBD. Residues Gln286, Tyr327, Leu340, and His449 that interact with **3d** are highlighted in orange. Hydrogen bonds are shown as yellow dotted lines. Tyr473 is also shown for reference. The water molecule interacting with Leu340 and the pyrazole nitrogen is depicted as a red sphere. Key hydrogen bonds are shown as yellow dotted lines. (B) Superposition of the docking model of the carboxylic-acid-based agonist **1** (shown in light purple) bound to the PPARγ-LBD [PDB code: 1FM6] and the co-crystal structure of **3d** (shown in white). Tyr473 interacting with the carboxyl group of compound **1** is highlighted in light purple. The conformational change of Phe363 is marked by a red circle. The hydrogen bond between Tyr473 and the carboxylic acid of **1** is shown as a yellow dotted line.

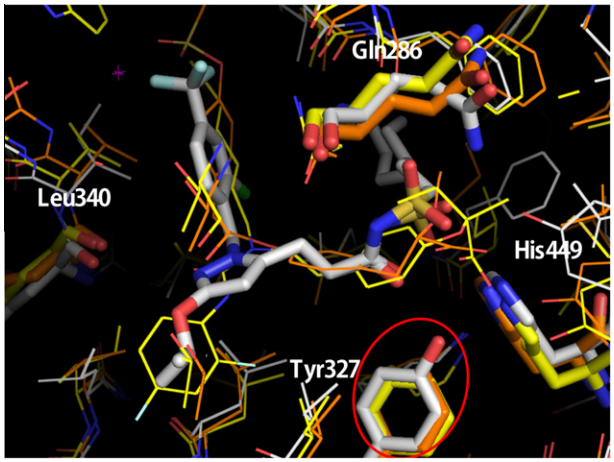


Figure 5. Superposition of the co-crystal structure of the benzylpyrazole acylsulfonamide **3d** bound to PPARγ-LBD (shown in white), PPARα/γ agonist AZ242 bound to PPARα-LBD (shown in orange) [PDB code: 1I7G], and PPARδ agonist GW2331 bound to PPARδ-LBD (shown in yellow) [PDB code: 1Y0S]. Residues Gln286, Tyr327, Leu340, and His449 in PPARγ that interact with **3d** are highlighted in white. The corresponding residues in PPARα and δ are highlighted in orange and yellow, respectively. The key replacement is marked by a red circle.

One of the most attractive features of the benzylpyrazole acylsulfonamides is high selectivity for PPARγ over the PPARα and δ subtypes. To examine the high selectivity profile of these compounds, the co-crystal structure of **3d** was superposed with the complex of PPARα-LBD with PPARα/δ dual agonist AZ242 (PDB code: 1I7G)[19] and PPARδ-LBD with PPARδ agonist GW2331 (PDB code: 1Y0S). [20] As mentioned above, the key interactions of **3d** with PPARγ-LBD are through direct or indirect hydrogen bonds with Gln286, Leu340, Tyr327, and His449. As shown in Figure 5, Leu340, Gln286, and His449 are conserved both in PPARα (Gln277, Leu331, and His440) and PPARδ (Gln286, Leu340, and His449).[21] However, Tyr327 in PPARγ is replaced with Phe318 and Phe327 in PPARα and PPARδ, respectively. Although the precise reason for the high selectivity remains unclear, but we speculate that the lack of interaction corresponding to the hydrogen bond with Tyr327 might influence the selectivity.

5. Conclusion

Herein, we described the design, synthesis, and in vitro and in vivo activities of novel benzylpyrazole acylsulfonamides as non-carboxylic-acid-based selective PPARγ agonists. Our strategy was based on the assumption that incorporation of an isosteric substitute of the carboxylic acid of the weak agonist **2**, which enhances the interaction with the TZD pocket, would afford non-carboxylic-acid-based PPARγ agonists. The lead compound **3c** was identified by incorporating acylsulfonamide, a ring-opening analog of TZD, into compound **2**. The optimization study revealed the following SAR findings: (i) incorporating an acylsulfonamide with a linear alkyl side chain, 2-carbon spacer, and 3-substituent on the pyrazole ring are important for potent PPARγ agonism, (ii) substitution of a trifluoromethyl group for the para-chlorine atom of the benzyl moiety tends to enhance in vitro potency, (iii) incorporation of a double bond into the ethylene spacer significantly improves metabolic stability. The PPARγ agonists **30b**, and **30c** with sufficient plasma exposure and potent antidiabetic efficacy were identified. The docking study indicated that the carboxylic-acid-based agonist **1** directly interacts with Tyr473. However, X-ray co-crystallographic analysis indicates that the acylsulfonamide-based agonist **3d** effectively occupies the Y-shaped cavity of the PPARγ-LBD without interacting with Tyr473. The conformational change of Phe363 creates the new cleft-like binding space to accommodate the terminal pentyl group, which could compensate for the decreased binding energy. Furthermore, the acylsulfonamide group of **3d** forms hydrogen bonds with the amino acid residues in TZD pocket, namely, Gln286, Try327, and His449. Among these interactions, it is speculated that hydrogen bonding with Tyr327 is the key interaction that might contribute to the high subtype selectivity. These results suggest that the benzylpyrazole acylsulfonamides are a new class of potent and highly selective PPARγ agonists that do not possess TZD structure or a carboxylic acid group.

6. Experimental section

6.1. Chemistry

6.1.1. General methods

Melting points (mp) were determined on a Büch melting point B-545 or a Yanagimoto micro melting point apparatus and were uncorrected. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AVANCE 300 (300 MHz) spectrometer or a Varian MERCURY plus 300 (300 MHz) spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. All *J* values are given in hertz. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublets of doublet; br s, broad singlet. Elemental analyses (C, H, N) were within ±0.3% of theoretical values and were determined in Takeda Analytical Research Laboratories (Osaka, Japan). Liquid Chromatography–Mass Spectrometry (LC–MS) analysis was performed on a Shiseido CAPCELL PACK C-18 UG120 S-3 column in a Waters Alliance 2795 or an Agilent 1100 System equipped with a Waters 2487 absorbance detector and a Micromass ZQ2000 mass spectrometer, eluted with water containing 0.05% TFA with a linear gradient of 10%–100% of MeCN containing 0.04% TFA. Mass spectra were recorded using electrospray ionization (ESI) in positive ion mode. Flash chromatography was performed with Merck silica gel 60 (0.063–0.200 mm) or Fuji Silysia Chemical Purif-Pack SI (60 μM) or Varian Bond Elut Si (40 μM). High-performance liquid chromatography (HPLC) was performed with Nomura Chemical Co., Ltd. Develosil ODS-UG10 using a Waters HPLC module equipped with a 2487 2-channel UV/VIS detector. Reaction progress was determined by thin layer chromatography (TLC) analysis on Merck silica gel 60 F_{254} plates. Visualization was with UV light (254 nm) or iodine. Yields are of purified compounds and were not optimized. Reagents and solvents were obtained from commercial sources and used without further purification. The following abbreviations are used: AcOH, acetic acid; ADDP, 1,1′-(azodicarbonyl)dipiperidine; CDI, *N,N*-carbonyldiimidazole; DMA, *N,N*-dimethylacetamide; DMF, *N,N*-dimethylformamide; DBU, 1,8-diazabicyclo[5.4.0]-7-undecene; DCM, dichloromethane; DIBAL-H, diisobutylaluminium hydride; DMAP, 4-dimethylaminopyridine; DMSO, dimethylsulfoxide; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; EtOAc, ethyl acetate; EtOH, ethanol; Et_2O, diethyl ether; IPE, diisopropyl ether; HOBt, 1-hydroxybenzotriazole; MeOH, methanol; TEA, triethylamine; THF, tetrahydrofuran.

6.1.1.1. Methyl 3-hydroxy-1*H*-pyrazole-5-carboxylate (4). To a solution of hydrazine monohydrate (44.8 g, 894 mmol) in toluene (300 mL) was added AcOH (180 mL) followed by addition of acetylenedicarboxylic acid dimethyl ester (100 mL, 813 mmol). After being stirred at room temperature for 2 h, the mixture was poured into ice-cooled water. The resulting precipitate was collected, washed with water and hexane, and dried to give **4** (92.2 g, 80%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 3.77 (s, 3H), 5.96 (br s, 1H), 10.04 (br s, 1H), 12.79 (br s, 1H).

6.1.1.2. Methyl 3-isopropoxy-1*H*-pyrazole-5-carboxylate (5). To a mixture of **4** (92.2 g, 649 mmol), K_2CO_3 (135 g, 974 mmol), and DMF (500 mL) was added 2-iodopropane (132 g, 779 mmol). After being stirred at room temperature for 15 h, the mixture was poured into water and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 100:1 to 1:1) to give **5** (105 g, 88%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.36 (d, J = 6.0 Hz, 6H), 3.91 (s, 3H), 4.67–4.83 (m, 1H), 6.20 (s, 1H), 10.12 (br s, 1H).

6.1.1.3. Methyl 1-benzyl-3-isopropoxy-1*H*-pyrazole-5-carboxylate (6). To a mixture of **5** (105 g, 573 mmol), K_2CO_3 (119 g, 860 mmol), and DMF (400 mL) was added benzyl bromide (117 g, 687 mmol). After being stirred at room temperature for 15 h, the mixture was poured into water and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 100:1 to 4:1) to give **6** (130 g, 83%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.34 (d, J = 6.3 Hz, 6H), 3.82 (s, 3H), 4.67–4.77 (m, 1H), 5.60 (s, 2H), 6.21 (s, 1H), 7.21–7.39 (m, 5H).

6.1.1.4. 1-Benzyl-3-isopropoxy-1*H*-pyrazole-5-carbaldehyde (7). To an ice-cooled solution of **6** (120 g, 438 mmol) in THF (1200 mL) was added 1.5 M DIBAL-H in toluene (729 mL, 1090 mmol). The solution was allowed to warm to room temperature, and stirred for 2 h. The reaction was quenched by successive addition of MeOH and 10% Rochelle salt, and the mixture was stirred vigorously until the white slurry was dissolved. The solution was extracted with Et_2O and the extract was washed with brine. The solution was dried over $MgSO_4$ and concentrated under reduced pressure. The crude products were purified by silica gel chromatography (hexane-EtOAc, 19:1 to 1:1) to give (1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)methanol (77.7 g, 72%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.33 (d, J = 6.0 Hz, 6H), 1.70 (t, J = 6.0 Hz, 1H), 4.47 (d, J = 6.0 Hz, 2H), 4.64–4.74 (m, 1H), 5.22 (s, 2H), 5.64 (s, 1H), 7.09–7.16 (m, 2H), 7.20–7.37 (m, 3H). To a solution of oxalyl chloride (80.0 g, 630 mmol) in DCM (400 mL) at −78 °C was added DMSO (73.8 g, 945 mmol). After being stirred for 10 min, a solution of the obtained intermediate (77.7 g, 315 mmol) in DCM (250 mL) was added. After being stirred for 1 h, TEA (159 g, 1.58 mol) was added. The mixture was allowed to warm to room temperature, and the stirring was continued for 1 h. The mixture was concentrated in vacuo and the residue was diluted with IPE. The insoluble materials were removed by filtration and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 19:1 to 4:1) to give **7** (76.8 g, 99%) as a brown oil. ^{1}H NMR ($CDCl_3$): δ 1.35 (d, J = 6.0 Hz, 6H), 4.69–4.81 (m, 1H), 5.55 (s, 2H), 6.24 (s, 1H), 7.08–7.15 (m, 5H), 9.70 (s, 1H).

6.1.1.5. Ethyl 3-(3-isopropoxy-1*H*-pyrazol-5-yl)propanoate (8). To an ice-cooled solution of ethyl diethylphosphonoacetate (91.4 g, 408 mmol) in DMF (300 mL) was added 60% NaH (16.3 g, 408 mmol). The mixture was allowed to warm to room temperature, and stirred for 30 min. After the mixture was cooed to 0 °C, a solution of **7** (76.8 g, 314 mmol) in THF (250 mL) was added. After being stirred for 1 h, the reaction was allowed to warm to room temperature, and the stirring was continued for 30 min. The mixture was concentrated in vacuo and partitioned between EtOAc and water. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 80:1 to 1:1) to give ethyl (*E*)-3-(1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)propenoate (83.9 g, 85%) as a brown oil. ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 3H), 1.34 (d, J = 6.3 Hz, 6H), 4.21 (q, J = 7.2 Hz, 2H), 4.65–4.77 (m, 1H), 5.27 (s, 2H), 5.96 (s, 1H), 6.23 (d, J = 15.8 Hz, 1H), 7.10–7.15 (m, 2H), 7.20–7.33 (m, 3H), 7.45 (d, J = 15.8 Hz, 1H). To a solution of the obtained intermediate (83.9 g, 268 mmol) in EtOH (700 mL) was added 10% Pd/C (100 g) followed by formic acid (700 mL). The mixture was heated under reflux for 15 h. After being cooled, the catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was partitioned between EtOAc and water. The organic layer was washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure to give a pale yellow oil. The oil was dissolved in EtOH (800 mL) and subjected to the same operations with 10% Pd/C (180 g) and formic acid (800 mL). After being cooled, the catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was partitioned between EtOAc and water. The organic layer was washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure to give **8** (49.2 g, 81%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.26 (t, J = 7.2 Hz, 3H), 1.33 (d, J = 6.3 Hz, 6H), 2.58–2.65 (m, 2H), 2.82–2.90 (m, 2H), 4.15 (q, J = 7.2 Hz, 2H), 4.64–4.76 (m, 1H), 5.46 (s, 1H).

6.1.1.6. Ethyl 3-[1-(2,4-dichlorobenzyl)-3-isopropoxy-1*H*-pyrazol-5-yl]propanoate (9a). To a solution of **8** (10.0 g, 44.2 mmol) in DMF (150 mL) was added 60% NaH (1.93 g, 48.3 mmol). After being stirred at room temperature for 10 min, 2,4-dichlorobenzyl chloride (13.0 g, 66.3 mmol) was added. After being stirred at room temperature for 15 h, the mixture was poured into sat. NH_4Cl and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc, 50:1 to 6:1) to give **9a** (11.3 g, 66%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.24 (t, J = 7.2 Hz, 3H), 1.32 (d, J = 6.3 Hz, 6H), 2.51–2.61 (m, 2H), 2.71–2.81 (m, 2H), 4.12 (q, J = 7.2 Hz, 2H), 4.64–4.77 (m, 1H), 5.18 (s, 2H), 5.52 (s, 1H), 6.56 (d, J = 8.4 Hz, 1H), 7.14 (dd, J = 2.1, 8.4 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H).

6.1.1.7. Ethyl 3-{1-[2-chloro-4-(trifluoromethyl)benzyl]-3-isopropoxy-1*H*-pyrazol-5-yl}propanoate (9b). Compound **9b** was prepared in 64% yield by a method similar to that described for **9a**. ^{1}H NMR ($CDCl_3$): δ 1.19–1.28 (t, J = 7.1 Hz, 3H), 1.33 (d, J = 6.3 Hz, 6H), 2.58 (t, J = 7.4 Hz, 2H), 2.77 (t, J = 7.4 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 4.65–4.76 (m, 1H), 5.26 (s, 2H), 5.55 (s, 1H), 6.70 (d, J = 8.0 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.64 (s, 1H).

6.1.1.8. 3-[1-(2,4-Dichlorobenzyl)-3-isopropoxy-1*H*-pyrazol-5-yl]-*N*-(pentylsulfonyl)propanamide (3c). A mixture of **9a** (4.00 g, 12.1 mmol), 1 M NaOH (18 mL, 18.0 mmol), THF (20 mL), and MeOH (20 mL) was heated at 50 °C with stirring for 2 h. After being cooled, the mixture was acidified with 1 M HCl (19 mL), diluted with brine, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was recrystallized from EtOAc/hexane to give 3-[1-(2,4-dichlorobenzyl)-3-isopropoxy-1*H*-pyrazol-5-yl]propanoic ac id (**2**) (3.54 g, 82%) as colorless crystals: mp 114–115 °C. ^{1}H NMR ($CDCl_3$): δ 1.33 (d, J = 6.2 Hz, 6H), 2.57–2.84 (m, 4H), 4.64–4.77 (m, 1H), 5.19 (s, 2H), 5.54 (s, 1H), 6.56 (d, J = 8.1 Hz, 1H), 7.14 (dd, J = 2.1, 8.1 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H). Anal. Calcd for $C_{16}H_{18}Cl_2N_2O_3$: C, 53.79; H, 5.08; N, 7.84. Found: C, 53.97; H, 5.12; N, 7.89. A mixture of **2** (500 mg, 1.40 mmol), CDI (341 mg, 2.10 mmol), and THF (10 mL) was heated under reflux with stirring for 1 h. After being cooled, 1-pentanesulfonamide (254 mg, 1.68 mmol) and DBU (320 mg, 2.10 mmol) were successively added. After being stirred at room temperature for 15 h, the mixture was concentrated in vacuo, acidified with 1 M HCl, and extracted with EtOAc. The extract was washed with brine, dried

over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 19:1 to 1:1) and successively recrystallized from EtOAc/hexane to give **3c** (450 mg, 66%) as colorless crystals: mp 126–129 °C. ^{1}H NMR ($CDCl_3$): δ 0.91 (t, J = 7.2 Hz, 3H), 1.24–1.48 (m, 10H), 1.74–1.86 (m, 2H), 2.54–2.62 (m, 2H), 2.77–2.85 (m, 2H), 3.34–3.41 (m, 2H), 4.63–4.76 (m, 1H), 5.18 (s, 2H), 5.50 (s, 1H), 6.57 (d, J = 8.1 Hz, 1H), 7.15 (dd, J = 2.1, 8.1 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H), 8.00 (br s, 1H). Anal. Calcd for $C_{21}H_{29}Cl_2N_3O_4S$: C, 51.43; H, 5.96; N, 8.57. Found: C, 51.32; H, 5.92; N, 8.69.

6.1.1.9. 3-{1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-isopropoxy-1*H*-pyrazol-5-yl}-*N*-(pentylsulfonyl)propanamide (3d). 3-{1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-isopropoxy-1*H*-pyrazol-5-yl} propanoic acid was prepared in 77% yield by a method similar to that described for **2**. Mp 121–122 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.33 (d, J = 6.0 Hz, 6H), 2.62–2.71 (m, 2H), 2.77 (t, J = 7.1 Hz, 2H), 4.63–4.79 (m, 1H), 5.27 (s, 2H), 5.57 (s, 1H), 6.71 (d, J = 8.1 Hz, 1H), 7.43 (d, J = 8.1 Hz, 1H), 7.63 (s, 1H). Anal. Calcd for $C_{17}H_{18}ClF_3N_2O_3$: C, 52.25; H, 4.64; N, 7.17. Found: C, 52.31; H, 4.72; N, 7.15. Compound **3d** was prepared in 6% yield from the corresponding carboxylic acid by a method similar to that described for **3c**. Mp 147–149 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.90 (t, J = 7.1 Hz, 3H), 1.32 (d, J = 6.2 Hz, 6H), 1.34–1.49 (m, 4H), 1.72–1.86 (m, 2H), 2.62 (t, J = 7.2 Hz, 2H), 2.82 (t, J = 7.3 Hz, 2H), 3.31–3.45 (m, 2H), 4.60–4.82 (m, 1H), 5.27 (s, 2H), 5.53 (s, 1H), 6.72 (d, J = 8.1 Hz, 1H), 7.44 (d, J = 0.9, 8.1 Hz, 1H), 7.64 (d, J = 0.9 Hz, 1H). Anal. Calcd for $C_{22}H_{29}ClF_3N_3O_4S$: C, 50.43; H, 5.58; N, 8.02. Found: C, 50.48; H, 5.60; N, 7.93.

6.1.1.10. *N*-(Butylsulfonyl)-3-[1-(2,4-dichlorobenzyl)-3-isopropoxy-1*H*-pyrazol-5-yl]propanamide (3e). Compound **3e** was prepared in 71% yield by a method similar to that described for **3c**. Mp 133–134 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.95 (t, J = 7.3 Hz, 3H), 1.32 (d, J = 6.2 Hz, 6H), 1.40–1.55 (m, 2H), 1.70–1.83 (m, 2H), 2.54–2.62 (m, 2H), 2.78–2.87 (m, 2H), 3.35–3.43 (m, 2H), 4.64–4.77 (m, 1H), 5.19 (s, 2H), 5.51 (s, 1H), 6.60 (d, J = 8.3 Hz, 1H), 7.16 (dd, J = 2.1, 8.3 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H), 7.80 (br s, 1H). Anal. Calcd for $C_{20}H_{27}Cl_2N_3O_4S$: C, 50.42; H, 5.71; N, 8.82. Found: C, 50.42; H, 5.64; N, 8.84.

6.1.1.11. 3-[1-(2,4-Dichlorobenzyl)-3-isopropoxy-1*H*-pyrazol-5-yl]-*N*-(hexylsulfonyl)propanamide (3f). Compound **3f** was prepared in 48% yield by a method similar to that described for **3c**. Mp 103–106 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.89 (t, J = 6.9 Hz, 3H), 1.24–1.48 (m, 12H), 1.70–1.86 (m, 2H), 2.53–2.63 (m, 2H), 2.76–2.86 (m, 2H), 3.33–3.43 (m, 2H), 4.63–4.76 (m, 1H), 5.18 (s, 2H), 5.51 (s, 1H), 6.59 (d, J = 8.4 Hz, 1H), 7.15 (dd, J = 2.1, 8.4 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H), 8.30 (br s, 1H). Anal. Calcd for $C_{22}H_{31}Cl_2N_3O_4S$: C, 52.38; H, 6.19; N, 8.33. Found: C, 52.35; H, 6.19; N, 8.25.

6.1.1.12. 3-[1-(2,4-Dichlorobenzyl)-3-isopropoxy-1*H*-pyrazol-5-yl]-*N*-[(3-methylbutyl)sulfonyl]propanamide (3g). Compound **3g** was prepared in 73% yield by a method similar to that described for **3c**. Mp 144 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.92 (d, J = 6.0 Hz, 6H), 1.32 (d, J = 6.3 Hz, 6H), 1.66–1.77 (m, 3H), 2.54–2.63 (m, 2H), 2.76–2.86 (m, 2H), 3.35–3.44 (m, 2H), 4.64–4.77 (m, 1H), 5.19 (s, 2H), 5.51 (s, 1H), 6.59 (d, J = 8.4 Hz, 1H), 7.15 (dd, J = 2.1, 8.4 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H), 7.95 (br s, 1H). Anal. Calcd for $C_{21}H_{29}Cl_2N_3O_4S$: C, 51.43; H, 5.96; N, 8.57. Found: C, 51.29; H, 5.79; N, 8.41.

6.1.1.13. *N*-(Cyclohexylsulfonyl)-3-[1-(2,4-dichlorobenzyl)-3-isopropoxy-1*H*-pyrazol-5-yl]propanamide (3h). Compound **3h** was prepared in 55% yield by a method similar to that described for **3c**. Mp 137–138 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.09–1.77 (m, 12H), 1.85–1.97 (m, 2H), 2.05–2.16 (m, 2H), 2.53–2.64 (m, 2H), 2.78–2.86 (m, 2H), 3.37–3.50 (m, 1H), 4.63–4.76 (m, 1H), 5.19 (s, 2H), 5.51 (s, 1H), 6.59 (d, J = 8.3 Hz, 1H), 7.16 (dd, J = 2.1, 8.3 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H), 7.90 (br s, 1H). Anal. Calcd for $C_{22}H_{29}Cl_2N_3O_4S$: C, 52.59; H, 5.82; N, 8.36. Found: C, 52.53; H, 5.73; N, 8.41.

6.1.1.14. 3-[1-(2,4-Dichlorobenzyl)-3-isopropoxy-1*H*-pyrazol-5-yl]-*N*-(phenylsulfonyl)propanamide (3i). Compound **3i** was prepared in 77% yield by a method similar to that described for **3c**. Mp 184–185 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.30 (d, J = 6.0 Hz, 6H), 2.47–2.54 (m, 2H), 2.67–2.75 (m, 2H), 4.58–4.70 (m, 1H), 5.08 (s, 2H), 5.36 (s, 1H), 6.48 (d, J = 8.3 Hz, 1H), 7.10 (dd, J = 2.1, 8.3 Hz, 1H), 7.34 (d, J = 2.1 Hz, 1H), 7.51–7.59 (m, 2H), 7.62–7.69 (m, 1H), 7.97–8.04 (m, 2H). Anal. Calcd for $C_{22}H_{23}Cl_2N_3O_4S$: C, 53.23; H, 4.67; N, 8.46. Found: C, 53.13; H, 4.58; N, 8.56.

6.1.1.15. *N*-(Benzylsulfonyl)-3-[1-(2,4-dichlorobenzyl)-3-isopropoxy-1*H*-pyrazol-5-yl]propanamide (3j). Compound **3j** was prepared in 87% yield by a method similar to that described for **3c**. Mp 183–184 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.32 (d, J = 6.0 Hz, 6H), 2.41–2.47 (m, 2H), 2.73–2.80 (m, 2H), 4.58 (s, 2H), 4.64–4.77 (m, 1H), 5.18 (s, 2H), 5.47 (s, 1H), 6.56 (d, J = 8.4 Hz, 1H), 7.11 (dd, J = 2.0, 8.4 Hz, 1H), 7.20–7.27 (m, 2H), 7.30–7.40 (m, 4H). Anal. Calcd for $C_{23}H_{25}Cl_2N_3O_4S$: C, 54.12; H, 4.94; N, 8.23. Found: C, 54.20; H, 4.92; N, 8.30.

6.1.1.16. Methyl 2-(2,4-dichlorobenzyl)hydrazinecarboxylate (11). A mixture of 2,4-dichlorobenzyl chloride **10** (108 g, 555 mmol), methyl carbazate (75.0 g, 833 mmol), K_2CO_3 (84.4 g, 611 mmol), and DMF (600 mL) was heated at 80 °C with stirring for 4 h. After being cooled, the mixture was poured into water and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 9:1 to 1:1) to give **11** (76.7 g, 55%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 3.72 (s, 3H), 4.10 (d, J = 5.7 Hz, 2H), 4.33 (br s, 1H), 6.19 (br s, 1H), 7.23 (dd, J = 2.1, 8.4 Hz, 1H), 7.35 (d, J = 8.4 Hz, 1H), 7.40 (d, J = 2.1 Hz, 1H).

6.1.1.17. Methyl 1-(2,4-dichlorobenzyl)-3-hydroxy-1*H*-pyrazole-5-carboxylate (12). To a solution of **11** (76.7 g, 308 mmol) in MeOH (500 mL) was added dimethyl acetylenedicarboxylate (43.8 g, 308 mmol). The mixture was heated under reflux with stirring for 2 h. After being cooled, NaOMe (19.1 g, 354 mmol) was added to the mixture. The mixture was heated under reflux with stirring for 1 h. After being cooled, the mixture was poured into 1 M HCl. The precipitate was collected and dried to give **12** (61.0 g, 66%) as a dark yellow solid. ^{1}H NMR (DMSO-d_6): δ 3.77 (s, 3H), 5.58 (s, 2H), 6.16 (s, 1H), 6.73 (d, J = 8.4 Hz, 1H), 7.38 (dd, J = 2.1, 8.4 Hz, 1H), 7.65 (d, J = 2.1 Hz, 1H), 10.33 (br s, 1H).

6.1.1.18. 1-(2,4-Dichlorobenzyl)-3-(methoxymethoxy)-1*H*-pyrazole-5-carbaldehyde (13). To an ice-cooed mixture of **12** (42.0 g, 139 mmol) and K_2CO_3 (28.8 g, 208 mmol) in DMF (350 mL) was added a solution of chloromethyl methyl ether (16.7 g, 208 mmol) in THF (100 mL). The mixture was allowed to warm to room temperature and stirred for 15 h. The mixture was poured into water and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 100:1 to 8:1) to give methyl 1-(2,4-dichlorobenzyl)-3-(methoxymethoxy)-1*H*-pyrazole-5-carboxylate (34.4 g, 72%) as a pale orange oil. ^{1}H NMR ($CDCl_3$): δ 3.51 (s, 3H), 3.82 (s,

3H), 5.22 (s, 2H), 5.70 (s, 2H), 6.43 (s, 1H), 6.56 (d, *J* = 8.4 Hz, 1H), 7.11 (dd, *J* = 2.1, 8.4 Hz, 1H), 7.37 (d, *J* = 2.1 Hz, 1H). [1-(2,4-Dichlorobenzyl)-3-(methoxymethoxy)-1H-pyrazol-5-yl]methanol was prepared from the obtained methyl ester in 84% yield by a similar method that described for (1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)methanol (see Section 6.1.1.4.). ^{1}H NMR ($CDCl_3$): δ 1.98 (t, *J* = 5.9 Hz, 1H), 3.51 (s, 3H), 4.53 (d, *J* = 5.9 Hz, 2H), 5.19 (s, 2H), 5.29 (s, 2H), 5.83 (s, 1H), 6.68 (d, *J* = 8.4 Hz, 1H), 7.14 (dd, *J* = 2.1, 8.1 Hz, 1H), 7.38 (d, *J* = 2.1 Hz, 1H). Compound **13** was prepared from the obtained alcohol in 99% yield by a method similar to that described for **7**. ^{1}H NMR ($CDCl_3$): δ 3.53 (s, 3H), 5.25 (s, 2H), 5.67 (s, 2H), 6.48 (s, 1H), 6.66 (d, *J* = 8.4 Hz, 1H), 7.13 (dd, *J* = 2.1, 8.4 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H), 9.76 (s, 1H).

6.1.1.19. Ethyl (E)-3-[1-(2,4-dichlorobenzyl)-3-(methoxymethoxy)-1*H*-pyrazol-5-yl]propenoate (14). Compound **14** was prepared in 90% yield by a method similar to that described for ethyl (*E*)-3-(1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)propenoate (see Section 6.1.1.5.). Mp 96–97 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.31 (t, *J* = 7.2 Hz, 3H), 3.52 (s, 3H), 4.23 (q, *J* = 7.2 Hz, 2H), 5.22 (s, 2H), 5.35 (s, 2H), 6.15 (s, 1H), 6.30 (d, *J* = 15.6 Hz, 1H), 6.71 (d, *J* = 8.4 Hz, 1H), 7.15 (dd, *J* = 2.1, 8.4 Hz, 1H), 7.40 (d, *J* = 2.1 Hz, 1H), 7.41 (d, *J* = 15.6 Hz, 1H).

6.1.1.20 Ethyl 3-[1-(2,4-dichlorobenzyl)-3-(methoxymethoxy)-1*H*-pyrazol-5-yl]propanoate (15). A mixture of **14** (19.0 g, 49.3 mmol), 5% Pd/C (2.10 g), and THF (200 mL) was hydrogenated under atmospheric pressure at room temperature for 6 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to give **15** (19.1 g, quant.) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.25 (t, *J* = 7.2 Hz, 3H), 2.53–2.62 (m, 2H), 2.73–2.82 (m, 2H), 3.51 (s, 3H), 4.13 (q, *J* = 7.2 Hz, 2H), 5.20 (s, 2H), 5.21 (s, 2H), 5.66 (s, 1H), 6.60 (d, *J* = 8.4 Hz, 1H), 7.15 (dd, *J* = 2.1, 8.4 Hz, 1H), 7.38 (d, *J* = 2.1 Hz, 1H).

6.1.1.21. Ethyl 3-[1-(2,4-dichlorobenzyl)-3-hydroxy-1*H*-pyrazol-5-yl]propanoate (16). A mixture of **15** (19.1 g, 49.3 mmol), concd. HCl (0.4 mL), and EtOH (200 mL) was heated under reflux with stirring for 6 h. After being cooled, the mixture was concentrated in vacuo and the residue was diluted with IPE/hexane/EtOAc. The resultant precipitate was collected and dried to give **16** (16.2 g, 96%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.24 (t, *J* = 7.2 Hz, 3H), 2.51–2.63 (m, 2H), 2.68–2.80 (m, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 5.18 (s, 2H), 5.49 (s, 1H), 5.60 (br s, 1H), 6.67 (d, *J* = 8.1 Hz, 1H), 7.18 (dd, *J* = 2.1, 8.1 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H).

6.1.1.22. Ethyl 3-[3-butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]propanoate (17a). To a solution of **16** (1.88 g, 5.48 mmol), 1-butanol (609 mg, 8.22 mmol), and tributylphosphine (2.23 g, 11.0 mmol) in THF (110 mL) was added ADDP (2.78 g, 11.0 mmol) portionwise. After being stirred at room temperature for 15 h, the mixture was concentrated in vacuo and the residue was diluted with IPE. The insoluble material was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 19:1 to 17:3) to give **17a** (2.07 g, 95%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.95 (t, *J* = 7.3 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H), 1.38–1.54 (m, 2H), 1.66–1.78 (m, 2H), 2.52–2.64 (m, 2H), 2.72–2.82 (m, 2H), 4.09 (t, *J* = 6.7 Hz, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 5.19 (s, 2H), 5.54 (s, 1H), 6.54 (d, *J* = 8.3 Hz, 1H), 7.15 (dd, *J* = 2.1, 8.3 Hz, 1H), 7.38 (d, *J* = 2.1 Hz, 1H).

6.1.1.23. Ethyl 3-[1-(2,4-dichlorobenzyl)-3-(2-methoxyethoxy)-1*H*-pyrazol-5-yl]propanoate (17b). Compound **17b** was prepared in 73% yield by a method similar to that described for **17a**. ^{1}H NMR ($CDCl_3$): δ 1.24 (t, *J* = 7.2 Hz, 3H), 2.52–2.62 (m, 2H), 2.70–2.80 (m, 2H), 3.43 (s, 3H), 3.67–3.79 (m, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 4.24–4.30 (m, 2H), 5.18 (s, 2H), 5.59 (s, 1H), 6.51 (d, *J* = 8.4 Hz, 1H), 7.14 (dd, *J* = 2.1, 8.4 Hz, 1H), 7.38 (d, *J* = 2.1 Hz, 1H).

6.1.1.24. Ethyl 3-[3-(benzyloxy)-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]propanoate (17c). Compound **17c** was prepared in 40% yield by a method similar to that described for **17a**. ^{1}H NMR ($CDCl_3$): δ 1.23 (t, *J* = 7.2 Hz, 3H), 2.52–2.62 (m, 2H), 2.72–2.82 (m, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 5.17 (s, 2H), 5.21 (s, 2H), 5.58 (s, 1H), 6.52 (d, *J* = 8.1 Hz, 1H), 7.13 (dd, *J* = 2.1, 8.1 Hz, 1H), 7.30–7.47 (m, 6H).

6.1.1.25. Ethyl 3-[1-(2,4-dichlorobenzyl)-3-(pyridin-2-ylmethoxy)-1*H*-pyrazol-5-yl]propanoate (17d). To a mixture of **16** (1.00 g, 2.91 mmol) and K_2CO_3 (1.01 g, 7.28 mmol) in DMF (20 mL) was added 2-(chloromethyl)pyridine hydrochloride (573 mg, 3.49 mmol). After being stirred at room temperature for 15 h, the mixture was poured into water and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 19:1 to 2:1) to give **17d** (1.00 g, 79%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.24 (t, *J* = 7.2 Hz, 3H), 2.53–2.63 (m, 2H), 2.73–2.83 (m, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 5.19 (s, 2H), 5.29 (s, 2H), 5.63 (s, 1H), 6.54 (d, *J* = 8.4 Hz, 1H), 7.12 (dd, *J* = 2.1, 8.4 Hz, 1H), 7.18–7.24 (m, 1H), 7.38 (d, *J* = 2.1 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.66–7.73 (m, 1H), 8.57–8.62 (m, 1H).

6.1.1.26. 3-[3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]-*N*-(pentylsulfonyl)propanamide (18a). 3-[3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]propanoic acid was prepared in 88% yield by a method similar to that described for **2**. Mp 123–124 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.95 (t, *J* = 7.3 Hz, 3H), 1.37–1.53 (m, 2H), 1.64–1.78 (m, 2H), 2.58–2.67 (m, 2H), 2.70–2.82 (m, 2H), 4.09 (t, *J* = 6.6 Hz, 2H), 5.19 (s, 2H), 5.56 (s, 1H), 6.55 (d, *J* = 8.1 Hz, 1H), 7.14 (dd, *J* = 2.1, 8.1 Hz, 1H), 7.38 (d, *J* = 2.1 Hz, 1H). Compound **18a** was prepared from the corresponding carboxylic acid in 66% yield by a method similar to that described for **3c**. Mp 104–105 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.85–1.00 (m, 6H), 1.24–1.87 (m, 10H), 2.53–2.63 (m, 2H), 2.75–2.86 (m, 2H), 3.33–3.43 (m, 2H), 4.08 (t, *J* = 6.6 Hz, 2H), 5.19 (s, 2H), 5.53 (s, 1H), 6.56 (d, *J* = 8.5 Hz, 1H), 7.15 (dd, *J* = 2.2, 8.5 Hz, 1H), 7.39 (d, *J* = 2.2 Hz, 1H), 8.17 (br s, 1H). Anal. Calcd for $C_{22}H_{31}Cl_2N_3O_4S$: C, 52.38; H, 6.19; N, 8.33. Found: C, 52.22; H, 6.17; N, 8.36.

6.1.1.27. 3-[1-(2,4-Dichlorobenzyl)-3-(2-methoxyethoxy)-1*H*-pyrazol-5-yl]-*N*-(pentylsulfonyl)propanamide (18b). 3-[1-(2,4-Dichlorobenzyl)-3-(2-methoxyethoxy)-1*H*-pyrazol-5-yl]propanoic acid was prepared in 94% yield by a method similar to that described for **2**. Mp 111–113 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 2.60–2.67 (m, 2H), 2.73–2.80 (m, 2H), 3.43 (s, 3H), 3.68–3.74 (m, 2H), 4.25–4.30 (m, 2H), 5.19 (s, 2H), 5.61 (s, 1H), 6.52 (d, *J* = 8.4 Hz, 1H), 7.13 (dd, *J* = 2.1, 8.4 Hz, 1H), 7.38 (d, *J* = 2.1 Hz, 1H). Anal. Calcd for $C_{16}H_{18}Cl_2N_2O_4$: C, 51.49; H, 4.86; N, 7.51. Found: C, 51.23; H, 5.02; N, 7.36. Compound **18b** was prepared from the corresponding carboxylic acid in 30% yield by a method similar to that described for **3c**. Mp 108–112 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.90 (t, *J* = 6.9 Hz, 3H), 1.23–1.48 (m, 4H), 1.65–1.86 (m, 2H), 2.52–2.63 (m, 2H), 2.74–2.85 (m, 2H), 3.32–3.41 (m, 2H), 3.42 (s, 3H), 3.65–3.75 (m, 2H), 4.23–4.30 (m, 2H), 5.18 (s, 2H), 5.55 (s, 1H), 6.54 (d, *J* = 8.4 Hz, 1H), 7.14 (dd, *J* = 2.1, 8.4 Hz, 1H), 7.38 (d, *J* = 2.1 Hz, 1H), 8.35 (br s, 1H). Anal. Calcd for $C_{21}H_{29}Cl_2N_3O_5S$: C, 49.80; H, 5.77; N, 8.30. Found: C, 49.69; H, 5.72; N, 8.24.

6.1.1.28. 3-[3-(Benzyloxy)-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]-*N*-(pentylsulfonyl)propanamide (18c). 3-[3-(Benzyloxy)-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]propanoic acid was prepared in 88% yield by a method similar to that described for **2**. Mp 98–101 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 2.60–2.67 (m, 2H), 2.72–2.80 (m, 2H), 5.17 (s, 2H), 5.20 (s, 2H), 5.60 (s, 1H), 6.52 (d, J = 8.4 Hz, 1H), 7.12 (dd, J = 2.1, 8.4 Hz, 1H), 7.25–7.45 (m, 6H). Anal. Calcd for $C_{20}H_{18}Cl_2N_2O_3$: C, 59.27; H, 4.48; N, 6.91. Found: C, 59.05; H, 4.35; N, 6.75. Compound **18c** was prepared from the corresponding carboxylic acid in 56% yield by a method similar to that described for **3c**. Mp 140 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.89 (3H, t, J = 7.1 Hz), 1.27–1.46 (m, 4H), 1.72–1.85 (m, 2H), 2.52–2.63 (m, 2H), 2.76–2.86 (m, 2H), 3.33–3.43 (m, 2H), 5.17 (s, 2H), 5.20 (s, 2H), 5.57 (s, 1H), 6.55 (d, J = 8.1 Hz, 1H), 7.13 (dd, J = 2.1, 8.1 Hz, 1H), 7.28–7.45 (m, 6H), 8.00 (br s, 1H). Anal. Calcd for $C_{25}H_{29}Cl_2N_3O_4S$: C, 55.76; H, 5.43; N, 7.80. Found: C, 55.58; H, 5.39; N, 7.73.

6.1.1.29. 3-[1-(2,4-Dichlorobenzyl)-3-(pyridin-2-ylmethoxy)-1*H*-pyrazol-5-yl]-*N*-(pentylsulfonyl)propanamide hydrochloride (18d). A mixture was **17d** (1.00 g, 2.30 mmol), 1 M NaOH (5.0 mL, 5.00 mmol), THF (10 mL), and EtOH (10 mL) was heated at 50 °C with stirring for 1 h. After being cooled, the mixture was concentrated in vacuo and the residue was diluted with water. The insoluble product was collected by filtration and dried to give 3-[1-(2,4-dichlorobenzyl)-3-(pyridin-2-ylmethoxy)-1*H*-pyrazol-5-yl]propanoic acid (760 mg, 75%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 2.46–2.56 (m, 2H), 2.67–2.77 (m, 2H), 5.15 (s, 2H), 5.20 (s, 2H), 5.72 (s, 1H), 6.62 (d, J = 8.4 Hz, 1H), 7.27–7.34 (m, 1H), 7.35 (dd, J = 2.1, 8.4 Hz, 1H), 7.43 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 2.1 Hz, 1H), 7.77–7.84 (m, 1H), 8.51–8.56 (m, 1H), 12.30 (br s, 1H). To a solution of the obtained carboxylic acid (760 mg, 1.87 mmol) in DMF (15 mL) was added CDI (455 mg, 2.81 mmol). After being stirred at room temperature for 1 h, 1-pentanesulfonamide (297 mg, 1.96 mmol) and DBU (428 mg, 2.81 mmol) were successively added. The mixture was heated at 100 °C with stirring for 15 h. After being cooled, the mixture was concentrated in vacuo and the residue was diluted with 1 M HCl/EtOAc (10/1), which was stirred at room temperature for 2 h. The insoluble product was collected by filtration and dried to give **18d** (500 mg, 46%) as a white solid: mp 167–169 °C. ^{1}H NMR (DMSO-d_6): δ 0.81 (t, J = 7.2 Hz, 3H), 1.17–1.38 (m, 4H), 1.52–1.67 (m, 2H), 2.56–2.67 (m, 2H), 2.72–2.83 (m, 2H), 3.26–3.38 (m, 2H), 5.20 (s, 2H), 5.28 (s, 2H), 5.71 (s, 1H), 6.63 (d, J = 8.4 Hz, 1H), 7.35 (dd, J = 2.1, 8.4 Hz, 1H), 7.56–7.74 (m, 3H), 8.08–8.19 (m, 1H), 8.67 (d, J = 4.8 Hz, 1H), 11.71 (br s, 1H). Anal. Calcd for $C_{24}H_{28}Cl_2N_4O_4S{\cdot}HCl{\cdot}H_2O$: C, 48.53; H, 5.25; N, 9.47. Found: C, 48.80; H, 5.25; N, 9.47.

6.1.1.30. Ethyl 3-isopropyl-1*H*-pyrazole-5-carboxylate (20a). To a mixture of *t*-BuOK (92.0 g, 697 mmol) and THF (500 mL) was added a mixture of 3-methyl-2-butanone (50.0 g, 581 mmol) and diethyl oxalate (85.8 g, 587 mmol) dropwise over 1 h. The mixture was stirred at room temperature for 15 h to form ethyl 5-methyl-2,4-dioxohexanoate. Thereafter, AcOH (80 mL, 1.39 mol) and hydrazine hydrate (32.0 g, 639 mmol) were successively added. The mixture was heated under reflux with stirring for 2 h. After being cooled, the mixture was concentrated in vacuo and the residue was partitioned between EtOAc and water. The organic solution was washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 9:1 to 1:1) to give **20a** (57.7 g, 55%) as a brown oil. ^{1}H NMR ($CDCl_3$): δ 1.30 (d, J = 6.9 Hz, 6H), 1.39 (t, J = 7.2 Hz, 3H), 2.95–3.12 (m, 1H), 4.38 (q, J = 7.2 Hz, 2H), 6.64 (s, 1H), 10.30 (br s, 1H).

6.1.1.31. Ethyl 3-cyclopropyl-1*H*-pyrazole-5-carboxylate (20b). Compound **20b** was prepared in 60% yield (2 steps) by a method similar to that described for **20a**. Mp 99–100 °C (IPE). ^{1}H NMR ($CDCl_3$): δ 0.68–0.82 (m, 2H), 0.96–1.06 (m, 2H), 1.34 (t, J = 7.2 Hz, 3H), 1.88–2.04 (m, 1H), 4.36 (q, J = 7.2 Hz, 2H), 6.45 (s, 1H), 11.30 (br s, 1H).

6.1.1.32. Ethyl 3-phenyl-1*H*-pyrazole-5-carboxylate (20c). Compound **20c** was prepared in 81% yield (2 steps) by a method similar to that described for **20a**. ^{1}H NMR ($CDCl_3$): δ 1.36 (t, J = 7.2 Hz, 3H), 4.36 (q, J = 7.2 Hz, 2H), 7.10 (s, 1H), 7.30–7.46 (m, 3H), 7.71–7.80 (m, 2H), 11.86 (br s, 1H).

6.1.1.33. Ethyl 1-(2,4-dichlorobenzyl)-3-isopropyl-1*H*-pyrazole-5-carboxylate (21a). Compound **21a** was prepared in 54% yield by a method similar to that described for **6**. ^{1}H NMR ($CDCl_3$): δ 1.29 (d, J = 7.2 Hz, 9H), 1.30 (t, J = 7.2 Hz, 3H), 2.95–3.09 (m, 1H), 4.26 (q, J = 7.2 Hz, 2H), 5.77 (s, 2H), 6.34 (d, J = 8.4 Hz, 1H), 6.78 (s, 1H), 7.09 (dd, J = 2.1, 8.4 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H).

6.1.1.34. Ethyl 1-[2-chloro-4-(trifluoromethyl)benzyl]-3-isopropyl-1*H*-pyrazole-5-carboxylate (21b). Compound **21b** was prepared in 42% yield by a method similar to that described for **6**. ^{1}H NMR ($CDCl_3$): δ 1.29 (t, J = 7.1 Hz, 3H), 1.30 (d, J = 7.0 Hz, 6H), 2.91–3.10 (m, 1H), 4.26 (q, J = 7.1 Hz, 2H), 5.85 (s, 2H), 6.48 (d, J = 8.1 Hz, 1H), 6.80 (s, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.64 (s, 1H).

6.1.1.35. Ethyl 1-[2-chloro-4-(trifluoromethyl)benzyl]-3-cyclopropyl-1*H*-pyrazole-5-carboxylate (21c). Compound **21c** was prepared in 56% yield by a method similar to that described for **6**. ^{1}H NMR ($CDCl_3$): δ 0.72–0.80 (m, 2H), 0.92–1.02 (m, 2H), 1.29 (t, J = 7.2 Hz, 3H), 1.90–2.02 (m, 1H), 4.25 (q, J = 7.2 Hz, 2H), 5.82 (s, 2H), 6.53 (d, J = 8.1 Hz, 1H), 6.62 (s, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.64 (s, 1H).

6.1.1.36. Ethyl 1-(2,4-dichlorobenzyl)-3-phenyl-1*H*-pyrazole-5-carboxylate (21d). Compound **21d** was prepared in 78% yield by a method similar to that described for **6**. Mp 104–107 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.33 (t, J = 7.2 Hz, 3H), 4.31 (q, J = 7.2 Hz, 2H), 5.88 (s, 2H), 6.52 (d, J = 8.4 Hz, 1H), 7.10 (dd, J = 2.1, 8.5 Hz, 1H), 7.38–7.47 (m, 4H), 7.80–7.87 (m, 2H). Anal. Calcd for $C_{19}H_{16}Cl_2N_2O_2{\cdot}0.1H_2O$: C, 60.52; H, 4.33; N, 7.43. Found: C, 60.41; H, 4.22; N, 7.44.

6.1.1.37. Ethyl (E)-3-[1-(2,4-dichlorobenzyl)-3-isopropyl-1*H*-pyrazol-5-yl]propenoate (22a). [1-(2,4-Dichlorobenzyl)-3-isopropyl-1*H*-pyrazol-5-yl]methanol was prepared in 99% yield by a method similar to that described for (1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)methanol (see experimental procedure of **7**). ^{1}H NMR ($CDCl_3$): δ 1.27 (d, J = 6.9 Hz, 6H), 1.63 (t, J = 5.6 Hz, 1H), 2.92–3.06 (m, 1H), 4.57 (d, J = 5.6 Hz, 2H), 5.49 (s, 2H), 6.14 (s, 1H), 6.59 (d, J = 8.0 Hz, 1H), 7.39 (dd, J = 1.2, 8.0 Hz, 1H), 7.62 (d, J = 1.2 Hz, 1H). 1-(2,4-Dichlorobenzyl)-3-isopropyl-1*H*-pyrazole-5-carbaldehyde was prepared from the corresponding alcohol in quantitative yield by a method similar to that described for **7**. ^{1}H NMR ($CDCl_3$): δ 1.30 (d, J = 7.0 Hz, 6H), 2.98–3.12 (m, 1H), 5.75 (s, 2H), 6.43 (d, J = 8.4 Hz, 1H), 6.81 (s, 1H), 7.10 (dd, J = 2.1, 8.4 Hz, 1H), 7.40 (d, J = 2.1 Hz, 1H), 9.80 (s, 1H). Compound **22a** was prepared in 99% yield by a method similar to that described for ethyl (*E*)-3-(1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)propenoate (see Section 6.1.1.5.). ^{1}H NMR ($CDCl_3$): δ 1.28 (d, J = 6.9 Hz, 6H), 1.30 (t, J = 7.2 Hz, 3H), 2.92–3.03 (m, 1H), 4.22 (q, J = 7.2 Hz, 2H), 5.44 (s, 2H), 6.30 (d, J = 15.9 Hz, 1H), 6.48 (s, 1H), 6.49 (d, J = 8.4 Hz, 1H), 7.13 (dd, J = 2.1, 8.4 Hz, 1H), 7.40 (d, J = 2.1 Hz, 1H), 7.41 (d, J = 15.9 Hz, 1H).

6.1.1.38. Ethyl (E)-3-{1-[2-chloro-4-(trifluoromethyl)benzyl]-3-isopropyl-1*H*-pyrazol-5-yl}propenoate (22b). {1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-isopropyl-1*H*-pyrazol-5-yl}methanol was prepared in 99% yield by a method similar to that described for (1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)methanol (see Section 6.1.1.4.). ^{1}H NMR ($CDCl_3$): δ 1.29 (d, J = 6.9 Hz, 6H), 1.63 (t, J = 5.6 Hz, 1H), 2.92–3.06 (m, 1H), 4.57 (d, J = 5.6 Hz, 1H), 5.49 (s, 2H), 6.14 (s, 1H), 6.59 (d, J = 8.0 Hz, 1H), 7.39 (dd, J = 1.2, 8.0 Hz, 1H), 7.62 (d, J = 1.2 Hz, 1H). 1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-isopropyl-1*H*-pyrazole-5-carbaldehyde was prepared from the corresponding alcohol in 99% yield by a method similar to that described for **7**. ^{1}H NMR ($CDCl_3$): δ 1.31(d, J = 7.2 Hz, 6H), 2.98–3.13 (m, 1H), 5.82 (s, 2H), 6.52 (d, J = 8.1 Hz, 1H), 6.83 (s, 1H), 7.36 (d, J = 1.2, 8.1 Hz, 1H), 7.65 (d, J = 1.2 Hz, 1H), 9.79 (s, 1H). Compound **22b** was prepared from the corresponding aldehyde in 29% yield by a method similar to that described for ethyl (*E*)-3-(1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)propenoate (see Section 6.1.1.5.). ^{1}H NMR ($CDCl_3$): δ 1.28 (d, J = 6.9 Hz, 6H), 1.30 (t, J = 7.2 Hz, 3H), 2.93–3.07 (m, 1H), 4.21 (q, J = 7.2 Hz, 2H), 5.51 (s, 2H), 6.31 (d, J = 15.8 Hz, 1H), 6.50 (s, 1H), 6.60 (d, J = 7.8 Hz, 1H), 7.38 (d, J = 15.8 Hz, 1H), 7.40 (d, J = 7.8 Hz, 1H), 7.65 (s, 1H).

6.1.1.39. Ethyl (E)-3-{1-[2-chloro-4-(trifluoromethyl)benzyl]-3-cyclopropyl-1*H*-pyrazol-5-yl}propenoate (22c). {1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-cyclopropyl-1*H*-pyrazol-5-yl}methanol was prepared in 84% yield by a method similar to that described for (1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)methanol (see Section 6.1.1.4.). ^{1}H NMR ($CDCl_3$): δ 0.68–0.75 (m, 2H), 0.88–0.98 (m, 2H), 1.63 (t, J = 5.7 Hz, 1H), 1.88–1.99 (m, 1H), 4.55 (d, J = 5.7 Hz, 2H), 5.45 (s, 2H), 5.96 (s, 1H), 6.66 (d, J = 8.1 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H), 7.63 (s, 1H). 1-[2-Chloro-4-(trifluoromethyl) benzyl]-3-cyclopropyl-1*H*-pyrazole-5-carbaldehyde was prepared from the corresponding alcohol in 84% yield by a method similar to that described for **7**. Mp 94–96 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.75–0.83 (m, 2H), 0.95–1.05 (m, 2H), 1.93–2.06 (m, 1H), 5.80 (s, 2H), 6.58 (d, J = 8.1 Hz, 1H), 6.67 (s, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.65 (s, 1H), 9.77 (s, 1H). Compound **22c** was prepared from the corresponding aldehyde in 97% yield by a method similar to that described for ethyl (*E*)-3-(1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)propenoate (see Section 6.1.1.5.). ^{1}H NMR ($CDCl_3$): δ 0.72–0.80 (m, 2H), 0.92–1.01 (m, 2H), 1.30 (t, J = 7.2 Hz, 3H), 1.88–2.00 (m, 1H), 4.22 (q, J = 7.2 Hz, 2H), 5.49 (s, 2H), 6.26 (s, 1H), 6.29 (d, J = 15.8 Hz, 1H), 6.66 (d, J = 8.1 Hz, 1H), 7.38 (d, J = 15.8 Hz, 1H), 7.42 (d, J = 8.1 Hz, 1H), 7.66 (s, 1H).

6.1.1.40. Ethyl (E)-3-[1-(2,4-dichlorobenzyl)-3-phenyl-1*H*-pyrazol-5-yl]propenoate (22d). [1-(2,4-Dichlorobenzyl)-3-phenyl-1*H*-pyrazol-5-yl]methanol was prepared in 92% yield by a method similar to that described for (1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)methanol (see Section 6.1.1.4.). Mp 140–141 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.95 (t, J = 5.3 Hz, 1H), 4.60 (d, J = 5.3 Hz, 2H), 5.49 (s, 2H), 6.58 (s, 1H), 6.63 (d, J = 8.3 Hz, 1H), 7.11 (dd, J = 2.1, 8.3 Hz, 1H), 7.27–7.45 (m, 4H), 7.78 (dd, J = 1.5, 8.4 Hz, 2H). 1-(2,4-Dichlorobenzyl)-3-phenyl-1*H*-pyrazole-5-carbaldehyde was prepared from the corresponding alcohol in 67% yield by a method similar to that described for **7**. Mp 136 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 5.85 (s, 2H), 6.61 (dd, J = 2.1, 8.4 Hz, 1H), 7.11 (dd, J = 2.1, 8.4 Hz, 1H), 7.27 (s, 1H), 7.32–7.49 (m, 4H), 7.80–7.87 (m, 2H), 9.89 (s, 1H). Anal. Calcd for $C_{17}H_{12}Cl_2N_2O$: C, 61.65; H, 3.65; N, 8.46. Found: C, 61.39; H, 3.63; N, 8.42. Compound **22d** was prepared from the corresponding aldehyde in 95% yield by a method similar to that described for ethyl (*E*)-3-(1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)propenoate (see Section 6.1.1.5.). mp 123–124 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.32 (d, J = 7.2 Hz, 3H), 4.24 (q, J = 7.2 Hz, 2H), 5.55 (s, 2H), 6.39 (d, J = 15.9 Hz, 1H), 6.67 (d, J = 8.5 Hz, 1H), 6.95 (s, 1H), 7.13 (dd, J = 2.1, 8.5 Hz, 1H), 7.30–7.46 (m, 4H), 7.48 (d, J = 15.9 Hz, 1H), 7.77–7.84 (m, 2H). Anal. Calcd for $C_{21}H_{18}Cl_2N_2O_2$: C, 62.85; H, 4.52; N, 6.98. Found: C, 62.79; H, 4.64; N, 6.77.

6.1.1.41. Ethyl 3-[1-(2,4-dichlorobenzyl)-3-isopropyl-1*H*-pyrazol-5-yl]propanoate (23a). Compound **23a** was prepared in 89% yield by a method similar to that described for **15**. ^{1}H NMR ($CDCl_3$): δ 1.24 (t, J = 7.2 Hz, 3H), 1.26 (d, J = 6.9 Hz, 6H), 2.55–2.63 (m, 2H), 2.74–2.82 (m, 2H), 2.90–3.04 (m, 1H), 4.12 (q, J = 7.2 Hz, 2H), 5.33 (s, 2H), 5.94 (s, 1H), 6.42 (d, J = 8.4 Hz, 1H), 7.12 (dd, J = 2.1, 8.4 Hz, 1H), 7.37 (d, J = 2.1 Hz, 1H).

6.1.1.42. Ethyl 3-{1-[2-chloro-4-(trifluoromethyl)benzyl]-3-isopropyl-1*H*-pyrazol-5-yl}propanoate (23b). Compound **23b** was prepared in 84% yield by a method similar to that described for **15**. ^{1}H NMR ($CDCl_3$): δ 1.23 (t, J = 7.2 Hz, 3H), 1.26 (d, J = 7.2 Hz, 6H), 2.57–2.64 (m, 2H), 2.74–2.81 (m, 2H), 2.89–3.02 (m, 1H), 4.11 (q, J = 7.2 Hz, 2H), 5.38 (s, 2H), 5.96 (s, 1H), 6.51 (d, J = 8.1 Hz, 1H), 7.40 (dd, J = 1.2, 8.1 Hz, 1H), 7.63 (d, J = 1.2 Hz, 1H).

6.1.1.43. Ethyl 3-{1-[2-chloro-4-(trifluoromethyl)benzyl]-3-cyclopropyl-1*H*-pyrazol-5-yl}propanoate (23c). Compound **23c** was prepared by a method similar to that described for **15** and obtained in 72% yield (calculated from ^{1}H NMR) along with an inseparable byproduct, ethyl 3-{1-[2-chloro-4-(trifluoromethyl) benzyl]-3-propyl-1*H*-pyrazol-5-yl}propanoate. ^{1}H NMR ($CDCl_3$): δ 0.67–0.74 (m, 2H), 0.87–0.97 (m, 2H), 1.23 (t, J = 7.2 Hz, 3H), 1.85–1.97 (m, 1H), 2.53–2.63 (m, 2H), 2.72–2.82 (m, 2H), 4.11 (q, J = 7.2 Hz, 2H), 5.36 (s, 2H), 5.79 (s, 1H), 6.60 (d, J = 8.1 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H), 7.64 (s, 1H).

6.1.1.44. Ethyl 3-[1-(2,4-dichlorobenzyl)-3-phenyl-1*H*-pyrazol-5-yl]propanoate (23d). Compound **23d** was prepared in 77% yield by a method similar to that described for **15**. Mp 91–93 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.25 (t, J = 7.2 Hz, 3H), 2.61–2.70 (m, 2H), 2.81–2.90 (m, 2H), 4.13 (q, J = 7.2 Hz, 2H), 5.41 (s, 2H), 6.43 (s, 1H), 6.56 (d, J = 8.4 Hz, 1H), 7.12 (dd, J = 2.1, 8.4 Hz, 1H), 7.25–7.42 (m, 4H), 7.72–7.81 (m, 2H). Anal. Calcd for $C_{21}H_{20}Cl_2N_2O_2{\cdot}0.1H_2O$: C, 62.26; H, 5.03; N, 6.92. Found: C, 62.19; H, 4.96; N, 6.85.

6.1.1.453-[1-(2,4-Dichlorobenzyl)-3-isopropyl-1*H*-pyrazol-5-yl]-*N*-(pentylsulfonyl)propanamide (24a). 3-[1-(2,4-Dichlorobenzyl)-3-isopropyl-1*H*-pyrazol-5-yl]propanoic acid was prepared in 74% yield by a method similar to that described for **2**. Mp 105–108 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.25 (d, J = 6.9 Hz, 6H), 2.63–2.68 (m, 2H), 5.31 (s, 2H), 5.96 (s, 1H), 6.39 (d, J = 8.3 Hz, 1H), 7.13 (dd, J = 2.2, 8.3 Hz, 1H), 7.39 (d, J = 2.2 Hz, 1H). Anal. Calcd for $C_{16}H_{18}Cl_2N_2O_2$: C, 56.32; H, 5.32; N, 8.21. Found: C, 56.22; H, 5.35; N, 8.20. Compound **24a** was prepared from the corresponding carboxylic acid in 67% yield by a method similar to that described for **3c**. Mp 115–117 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.90 (t, J = 7.2 Hz, 3H), 1.25 (d, J = 6.9 Hz, 6H), 1.28–1.47 (m, 4H), 1.72–1.84 (m, 2H), 2.55–2.63 (m, 2H), 2.78–2.86 (m, 2H), 2.88–3.02 (m, 1H), 3.34–3.42 (m, 2H), 5.30 (s, 2H), 5.93 (s, 1H), 6.40 (d, J = 8.4 Hz, 1H), 7.12 (dd, J = 1.8, 8.4 Hz, 1H), 7.38 (d, J = 1.8 Hz, 1H), 8.20 (br s, 1H). Anal. Calcd for $C_{21}H_{29}Cl_2N_3O_3S$: C, 53.16; H, 6.16; N, 8.86. Found: C, 53.15; H, 6.17; N, 8.78.

6.1.1.46. 3-{1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-isopropyl-1*H*-pyrazol-5-yl}-*N*-(pentylsulfonyl)propanamide (24b). 3-{1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-isopropyl-1*H*-pyrazol-5-yl}propanoic acid was prepared in 68% yield by a method similar to that described for **2**. Mp 118 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.25 (t, J = 7.2 Hz, 6H), 2.51–2.53 (m, 2H), 2.54–2.60

(m, 2H), 2.90–3.03 (m, 1H), 5.40 (s, 2H), 5.98 (s, 1H), 6.53 (d, J = 8.0 Hz, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.63 (s, 1H). Anal. Calcd for $C_{17}H_{18}ClF_3N_2O_2$: C, 54.48; H, 4.84; N, 7.47. Found: C, 54.43; H, 4.85; N, 7.38. Compound **24b** was prepared from the corresponding carboxylic acid in 38% yield by a method similar to that described for **3c**. Mp 144–145 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.90 (t, J = 7.1 Hz, 3H), 1.26 (d, J = 7.0 Hz, 6H), 1.28–1.47 (m, 4H), 1.72–1.85 (m, 2H), 2.58–2.68 (m, 2H), 2.78–2.87 (m, 2H), 2.89–3.03 (m, 1H), 3.33–3.43 (m, 2H), 5.39 (s, 2H), 5.96 (s, 1H), 6.55 (d, J = 8.1 Hz, 1H), 7.42 (dd, J = 1.0, 8.1 Hz, 1H), 7.65 (d, J = 1.0 Hz, 1H), 8.10 (br s, 1H). Anal. Calcd for $C_{22}H_{29}ClF_3N_3O_3S$: C, 52.02; H, 5.75; N, 8.27. Found: C, 52.03; H, 5.76; N, 8.18.

6.1.1.47. 3-{1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-cyclopropyl-1*H*-pyrazol-5-yl}-*N*-(pentylsulfonyl)propanamide (24c). 3-{1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-cyclopropyl-1*H*-pyrazol-5-yl}propanoic acid was prepared by a method similar to that described for **2** and obtained in 22% yield along with the corresponding 3-propyl-1*H*-pyrazole derivative (see Section 6.1.1.43.). ^{1}H NMR ($CDCl_3$): δ 0.66–0.74 (m, 2H), 0.87–0.97 (m, 2H), 1.85–1.98 (m, 1H), 2.60–2.67 (m, 2H), 2.72–2.79 (m, 2H), 5.36 (s, 2H), 5.80 (s, 1H), 6.59 (d, J = 8.1 Hz, 1H), 7.40 (dd, J = 0.9, 8.1 Hz, 1H), 7.63 (d, J = 0.9 Hz, 1H). To a solution of the obtained carboxylic acid (450 mg, 1.21 mmol) in DMF (5 mL) was added CDI (216 mg, 1.33 mmol). After being stirred at room temperature for 1 h, 1-pentylsulfonamide (201 mg, 1.33 mmol) and DBU (0.199 mL, 1.33 mmol) were successively added. The mixture was heated at 100 °C with stirring for 12 h. After being cooled, the mixture was acidified (pH 4) with 1 M HCl, diluted with water, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 20:1 to 2:1) to give a mixture of **24c** and the corresponding 3-propyl-1*H*-pyrazole derivative. The mixture was separated using reversed-phase high-performance liquid chromatography (HPLC), eluted with water containing 0.01% TFA with a linear gradient of 0% to 40% of MeCN. Recrystallization from EtOAc/hexane gave **24c** (261 mg, 43%) as colorless crystals: mp 152–154 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.57–0.76 (m, 2H), 0.81–0.98 (m, 5H), 1.19–1.47 (m, 4H), 1.70–1.84 (m, 2H), 1.84–1.96 (m, 1H), 2.60 (t, J = 7.4 Hz, 2H), 2.81 (t, J = 7.3 Hz, 2H), 3.22–3.52 (m, 2H), 5.36 (s, 2H), 5.79 (s, 1H), 6.61 (d, J = 8.0 Hz, 1H), 7.42 (dd, J = 0.9, 8.2 Hz, 1H), 7.65 (d, J = 0.9 Hz, 1H), 8.07 (br s, 1H). ESI MS m/z 506.1 $[C_{22}H_{27}ClF_3N_3O_3S+H]^+$.

6.1.1.48. 3-[1-(2,4-Dichlorobenzyl)-3-phenyl-1*H*-pyrazol-5-yl]-*N*-(pentylsulfonyl)propanamide (24d). 3-[1-(2,4-Dichlorobenzyl)-3-phenyl-1*H*-pyrazol-5-yl]propanoic acid was prepared in 88% yield by a method similar to that described for **2**. Mp 160–161 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 2.67–2.75 (m, 2H), 2.81–2.89 (m, 2H), 5.42 (s, 2H), 6.45 (s, 1H), 6.57 (d, J = 8.7 Hz, 1H), 7.12 (dd, J = 2.1, 8.7 Hz, 1H), 7.26–7.43 (m, 4H), 7.75–7.82 (m, 2H). Compound **24d** was prepared in 11% yield by a method similar to that described for **3c**. Mp 145–147 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.85 (t, J = 7.2 Hz, 3H), 1.18–1.40 (m, 4H), 1.68–1.82 (m, 2H), 2.57–2.67 (m, 2H), 2.83–2.93 (m, 2H), 3.30–3.42 (m, 2H), 5.41 (s, 2H), 6.42 (s, 1H), 6.59 (d, J = 8.1 Hz, 1H), 7.12 (dd, J = 2.1, 8.1 Hz, 1H), 7.23–7.42 (m, 4H), 7.72–7.80 (m, 2H), 8.17 (br s, 1H).

6.1.1.49. (E)-3-[1-(2,4-Dichlorobenzyl)-3-isopropyl-1*H*-pyrazol-5-yl]-*N*-(pentylsulfonyl)propenamide (25a). 3-[1-(2,4-dichlorobenzyl)-3-isopropyl-1*H*-pyrazol-5-yl]propenoic acid was prepared in 75% yield by a method similar to that described for **2**. Mp 174–175 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.28 (d, J = 6.9 Hz, 6H), 2.93–3.08 (m, 1H), 5.46 (s, 2H), 6.29 (d, J = 15.7 Hz, 1H), 6.51 (d, J = 8.5 Hz, 1H), 6.53 (s, 1H), 7.14 (d, J = 2.2 Hz, 1H), 7.41 (d, J = 2.2 Hz, 1H), 7.49 (d, J = 15.7 Hz, 1H). Compound **25a** was prepared from the corresponding carboxylic acid in 24% yield by a method similar to that described for **3c**. Mp 150 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.89 (t, J = 7.2 Hz, 3H), 1.28 (d, J = 7.0 Hz, 6H), 1.29–1.46 (m, 4H), 1.76–1.88 (m, 2H), 2.93–3.05 (m, 1H), 3.41–3.50 (m, 2H), 5.46 (s, 2H), 6.28 (d, J = 15.3 Hz, 1H), 6.50 (d, J = 8.3 Hz, 1H), 6.51 (s, 1H), 7.13 (dd, J = 2.1, 8.3 Hz, 1H), 7.41 (d, J = 2.1 Hz, 1H), 7.56 (d, J = 15.3 Hz, 1H), 8.12 (br s, 1H). Anal. Calcd for $C_{21}H_{27}Cl_2N_3O_3S$: C, 53.39; H, 5.76; N, 8.89. Found: C, 53.14; H, 5.70; N, 8.87.

6.1.1.50. (E)-3-{1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-cyclopropyl-1*H*-pyrazol-5-yl}-*N*-(pentylsulfonyl)propenamide (25c). (*E*)-3-{1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-cyclopropyl-1*H*-pyrazol-5-yl}propenoic acid was prepared in 86% yield by a method similar to that described for **2**. Mp 191–192 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.71–0.80 (m, 2H), 0.91–1.03 (m, 2H), 1.88–2.02 (m, 1H), 5.50 (s, 2H), 6.27 (d, J = 15.8 Hz, 1H), 6.37 (s, 1H), 6.69 (d, J = 8.1 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H), 7.45 (d, J = 15.8 Hz, 1H), 7.66 (s, 1H). Anal. Calcd for $C_{17}H_{14}ClF_3N_2O_2$: C, 55.07; H, 3.81; N, 7.56. Found: C, 55.05; H, 3.84; N, 7.61. To a solution of the obtained carboxylic acid (501 mg, 1.35 mmol) in DMF (5 mL) was added CDI (242 mg, 1.49 mmol). After being stirred at room temperature for 1 h, 1-pentylsulfonamide (225 mg, 1.49 mmol) and DBU (0.223 mL, 1.49 mmol) were successively added. The mixture was heated at 100 °C with stirring for 12 h. After being cooled, the mixture was acidified (pH 4) with 1 M HCl, diluted with water, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 20:1 to 2:1) and successively recrystallized from EtOAc/hexane to give **25c** (313 mg, 46%) as colorless crystals: mp 139–141 °C. ^{1}H NMR ($CDCl_3$): δ 0.66–0.81 (m, 2H), 0.89 (t, J = 7.1 Hz, 3H), 0.93–1.05 (m, 2H), 1.19–1.50 (m, 4H), 1.71–1.88 (m, 2H), 1.88–2.03 (m, 1H), 3.25–3.58 (m, 2H), 5.50 (s, 2H), 6.28 (d, J = 15.3 Hz, 1H), 6.38 (s, 1H), 6.67 (d, J = 8.1 Hz, 1H), 7.42 (dd, J = 0.9, 8.1 Hz, 1H), 7.53 (d, J = 15.5 Hz, 1H), 7.66 (d, J = 0.9 Hz, 1H), 8.22 (br s, 1H). Anal. Calcd for $C_{22}H_{25}ClF_3N_3O_3S$: C, 52.43; H, 5.00; N, 8.34. Found: C, 52.38; H, 4.92; N, 8.37. ESI MS m/z 504.0 $[C_{22}H_{25}ClF_3N_3O_3S+H]^+$.

6.1.1.51. Methyl 1-[2-chloro-4-(trifluoromethyl)benzyl]-3-(methoxymethoxy)-1*H*-pyrazole-5-carboxylate (26). To an ice-cooled mixture of **4** (68.6 g, 483 mmol), K_2CO_3 (100 g, 727 mmol), and DMF (350 mL) was added chloromethyl methyl ether (46.7 g, 580 mmol). The mixture was allowed to warm to room temperature and stirred for 15 h. The mixture was poured into water and extracted with EtOAc. The aqueous layer was further extracted with chloroform. The combined organic extracts were concentrated in vacuo and the residual solid was dissolved in chloroform. The solution was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 24:1 to 2:1) and successively recrystallized from EtOAc/hexane to give methyl 3-(methoxymethoxy)-1*H*-pyrazole-5-carboxylate (23.4 g, 26%) as colorless crystals: mp 54–55 °C. ^{1}H NMR ($CDCl_3$): δ 3.52 (s, 3H), 3.91 (s, 3H), 5.26 (s, 2H), 6.35 (s, 1H), 10.22 (br s, 1H). Compound **26** was prepared in 71% yield by a method similar to that described for **6**. ^{1}H NMR ($CDCl_3$): δ 3.53 (s, 3H), 3.83 (s, 3H), 5.24 (s, 2H), 5.79 (s, 2H), 6.46 (s, 1H), 6.70 (d, J = 8.1 Hz, 1H), 7.40 (dd, J = 0.9, 8.1 Hz, 1H), 7.64 (d, J = 0.9 Hz, 1H).

6.1.1.52. 1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-(methoxymethoxy)-1*H*-pyrazole-5-carbaldehyde (27). 1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-(methoxymethoxy)-1*H*-pyrazole-5-car-

boxylic acid was prepared in 86% yield by a method similar to that described for **2**. Mp 133–134 °C. ^{1}H NMR ($CDCl_3$): δ 3.52 (s, 3H), 5.24 (s, 2H), 5.76 (s, 2H), 6.56 (s, 1H), 6.71 (d, J = 8.1 Hz, 1H), 7.40 (d, J = 8.1 Hz, 1H), 7.64 (s, 1H), 8.60 (br s, 1H). Anal. Calcd for $C_{14}H_{12}ClF_3N_2O_4$: C, 46.11; H, 3.32; N, 7.68. Found: C, 46.11; H, 3.27; N, 7.66. To a stirred mixture of *N*,*O*-dimethylhydroxylamine (9.01 g, 92.4 mmol), TEA (9.35 g, 92.4 mmol), and DMF (300 mL) were added the obtained carboxylic acid (28.1 g, 77.0 mmol), EDCI (17.7 g, 92.4 mmol), and HOBt (14.2 g, 92.4 mmol). After being stirred at room temperature for 15 h, the mixture was concentrated in vacuo and partitioned between EtOAc and water. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was diluted with EtOAc and the insoluble product was removed by filtration. The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc, 19:1 to 1:1) to give 1-[2-chloro-4-(trifluoromethyl)benzyl]-*N*-methoxy-3-(methoxymethoxy)-*N*-methyl-1*H*-pyrazole-5-carboxamide (29.1 g, 93%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 3.29 (s, 3H), 3.53 (s, 3H), 3.67 (s, 3H), 5.25 (s, 2H), 5.70 (s, 2H), 6.41 (s, 1H), 6.75 (d, J = 8.1 Hz, 1H), 7.40 (d, J = 8.1 Hz, 1H), 7.62 (s, 1H). To an ice-cooled solution of the obtained carboxamide (29.1 g, 71.4 mmol) in THF (500 mL) at −78 °C was added 1.5 M DIBAL-H in toluene (71 mL, 107 mmol). The mixture was stirred at 0 °C for 1 h. To the solution was further added 1.5 M DIBAL-H in toluene (10 mL, 6.67 mmol). After being stirred at the same temperature for 0.5 h, the reaction was quenched by successive addition of MeOH and 10% Rochelle. The mixture was extracted with Et_2O and the extract was washed with brine. The solution was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc, 19:1 to 2:1) to give **27** (21.2 g, 85%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 3.53 (s, 3H), 5.26 (s, 2H), 5.75 (s, 2H), 6.51 (s, 1H), 6.75 (d, J = 8.1 Hz, 1H), 7.40 (d, J = 8.1 Hz, 1H), 7.65 (s, 1H), 9.76 (s, 1H).

6.1.1.53. Ethyl (E)-3-[1-[2-chloro-4-(trifluoromethyl)benzyl]-3-(methoxymethoxy)-1*H*-pyrazol-5-yl]pro- penoate (28). Compound **28** was prepared in 60% yield by a method similar to that described for ethyl (*E*)-3-(1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)propenoate (see Section 6.1.1.5.). ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 3H), 3.53 (s, 3H), 4.23 (q, J = 7.2 Hz, 2H), 5.23 (s, 2H), 5.43 (s, 2H), 6.18 (s, 1H), 6.32 (d, J = 15.5 Hz, 1H), 6.83 (d, J = 8.1 Hz, 1H), 7.39 (d, J = 15.5 Hz, 1H), 7.43 (d, J = 8.1 Hz, 1H), 7.66 (s, 1H).

6.1.1.54. Ethyl (E)-3-[1-(2,4-dichlorobenzyl)-3-isopropoxy-1*H*-pyrazol-5-yl]propenoate (29a). Ethyl (*E*)-3-[1-(2,4-dichlorobenzyl)-3-hydroxy-1*H*-pyrazol-5-yl]propenoate was prepared in 95% yield by a method similar to that described for **16**. ^{1}H NMR ($CDCl_3$): δ 1.31 (t, J = 7.2 Hz, 3H), 4.23 (q, J = 7.2 Hz, 2H), 5.28 (s, 2H), 5.95 (s, 1H), 6.32 (d, J = 15.6 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 7.17 (dd, J = 2.1, 8.4 Hz, 1H), 7.38 (d, J = 15.6 Hz, 1H), 7.41 (d, J = 2.1 Hz, 1H), 10.75 (br s, 1H). Compound **29a** was prepared from the corresponding hydroxypyrazole in 87% yield by a method similar to that described for **17d**. ^{1}H NMR ($CDCl_3$): δ 1.31 (t, J = 7.1 Hz, 3H), 1.34 (d, J = 6.2 Hz, 6H), 4.23 (q, J = 7.1 Hz, 2H), 4.66–4.79 (m, 1H), 5.33 (s, 2H), 6.00 (s, 1H), 6.27 (d, J = 15.9 Hz, 1H), 6.66 (d, J = 8.3 Hz, 1H), 7.15 (dd, J = 2.1, 8.3 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H), 7.40 (d, J = 15.9 Hz, 1H).

6.1.1.55. Ethyl (E)-3-{1-[2-chloro-4-(trifluoromethyl)benzyl]-3-isopropoxy-1*H*-pyrazol-5-yl}propenoate (29b). Ethyl (*E*)-3-{1-[2-chloro-4-(trifluoromethyl)benzyl]-3-hydroxy-1*H*-pyrazol-5-yl} propenoate was prepared in 87% yield by a method similar to that described for **16**. ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 3H), 4.23 (q, J = 7.2 Hz, 2H), 5.36 (s, 2H), 5.98 (s, 1H), 6.34 (d, J = 15.8 Hz, 1H), 6.84 (d, J = 8.1 Hz, 1H), 7.36 (d, J = 15.8 Hz, 1H), 7.44 (d, J = 8.1 Hz, 1H), 7.67 (s, 1H). Compound **29b** was prepared from the corresponding hydroxypyrazole in 90% yield by a method similar to that described for **17d**. ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 3H), 1.34 (d, J = 6.3 Hz, 6H), 4.22 (q, J = 7.2 Hz, 2H), 4.67–4.81 (m, 1H), 5.40 (s, 2H), 6.03 (s, 1H), 6.29 (d, J = 15.6 Hz, 1H), 6.78 (d, J = 8.1 Hz, 1H), 7.38 (d, J = 15.6 Hz, 1H), 7.43 (d, J = 8.1 Hz, 1H), 7.65 (s, 1H).

6.1.1.56. Ethyl (E)-3-[1-[2-chloro-4-(trifluoromethyl)benzyl]-3-(2-methoxyethoxy)-1*H*-pyrazol-5-yl]propenoate (29c). Compound **29c** was prepared in quantitative yield by a method similar to that described for **17a**. Mp 68–69 °C (IPE/hexane). ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 3H), 3.44 (s, 3H), 3.63–3.79 (m, 2H), 4.22 (q, J = 7.2 Hz, 2H), 4.28–4.35 (m, 2H), 5.40 (s, 2 H), 6.09 (s, 1H), 6.30 (d, J = 15.7 Hz, 1H), 6.75 (d, J = 8.1 Hz, 1H), 7.37 (d, J = 15.7 Hz, 1H), 7.42 (d, J = 8.1 Hz, 1H), 7.66 (s, 1H). Anal. Calcd for $C_{19}H_{20}ClF_3N_2O_4$: C, 52.72; H, 4.66; N, 6.47. Found: C, 52.65; H, 4.73; N, 6.39.

6.1.1.57. (E)-3-[1-(2,4-Dichlorobenzyl)-3-isopropoxy-1*H*-pyrazol-5-yl]-*N*-(pentylsulfonyl)propenamide (30a). (*E*)-3-[1-(2,4-Dichlorobenzyl)-3-isopropoxy-1*H*-pyrazol-5-yl]propenoic acid was prepared in 90% yield by a method similar to that described for **2**. Mp 170–171 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.34 (d, J = 6.0 Hz, 6H), 4.66–4.79 (m, 1H), 5.34 (s, 2H), 6.04 (s, 1H), 6.26 (d, J = 15.6 Hz, 1H), 6.69 (d, J = 8.4 Hz, 1H), 7.16 (dd, J = 2.1, 8.4 Hz, 1H), 7.40 (d, J = 2.1 Hz, 1H), 7.48 (d, J = 15.6 Hz, 1H). Compound **30a** was prepared from the corresponding carboxylic acid in 69% yield by a method similar to that described for **25c**. Mp 132–133 °C. ^{1}H NMR ($CDCl_3$): δ 0.90 (t, J = 7.2 Hz, 3H), 1.22–1.47 (m, 10H), 1.76–1.89 (m, 2H), 3.42–3.50 (m, 2H), 4.67–4.80 (m, 1H), 5.34 (s, 2H), 6.03 (s, 1H), 6.25 (d, J = 15.4 Hz, 1H), 6.67 (d, J = 8.5 Hz, 1H), 7.16 (dd, J = 2.1, 8.5 Hz, 1H), 7.40 (d, J = 2.1 Hz, 1H), 7.54 (d, J = 15.4 Hz, 1H), 8.05 (br s, 1H). Anal. Calcd for $C_{21}H_{27}Cl_2N_3O_4S$: C, 51.64; H, 5.57; N, 8.60. Found: C, 51.69; H, 5.60; N, 8.59.

6.1.1.58. (E)-3-{1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-isopropoxy-1*H*-pyrazol-5-yl}-*N*-(pentylsulfonyl)propenamide (30b). (*E*)-3-{1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-isopropoxy-1*H*-pyrazol-5-yl}propenoic acid was prepared in 95% yield by a method similar to that described for **2**. Mp 170–171 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 1.35 (d, J = 6.3 Hz, 6H), 4.67–4.80 (m, 1H), 5.41 (s, 2H), 6.07 (s, 1H), 6.28 (d, J = 15.8 Hz, 1H), 6.81 (d, J = 8.1 Hz, 1H), 7.44 (d, J = 8.1 Hz, 1H), 7.46 (d, J = 15.8 Hz, 1H), 7.66 (s, 1H). Anal. Calcd for $C_{17}H_{16}ClF_3N_2O_3$: C, 52.52; H, 4.15; N, 7.21. Found: C, 52.54; H, 4.09; N, 7.10. Compound **30b** was prepared from the corresponding carboxylic acid in 31% yield by a method similar to that described for **25c**. Mp 139–140 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.89 (t, J = 7.4 Hz, 3H), 1.23–1.47 (m, 10H), 1.76–1.89 (m, 2H), 3.40–3.50 (m, 2H), 4.69–4.82 (m, 1H), 5.42 (s, 2H), 6.06 (s, 1H), 6.28 (d, J = 15.3 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 7.43 (dd, J = 0.9, 8.1 Hz, 1H), 7.53 (d, J = 15.3 Hz, 1H), 7.66 (d, J = 0.9 Hz, 1H), 8.05 (br s, 1H). Anal. Calcd for $C_{22}H_{27}ClF_3N_3O_4S{\cdot}0.3H_2O$: C, 50.10; H, 5.27; N, 7.97. Found: C, 50.06; H, 5.11; N, 7.79.

6.1.1.59. (E)-3-{1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-isopropoxy-1*H*-pyrazol-5-yl}-*N*-[(3-methylbutyl)sulfonyl]propenamide (30c). Compound **30c** was prepared in 41% yield by a method similar to that described for **25c**. Mp 138–140 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.92 (d, J = 6.6 Hz, 6H), 1.34 (d, J = 6.0 Hz, 6H), 1.64–1.78 (m, 3H), 3.40–3.53 (m, 2H), 4.69–4.82 (m, 1H), 5.42 (s, 2H), 6.06 (s, 1H), 6.27 (d, J = 15.2 Hz, 1H), 6.69 (d, J = 8.1 Hz, 1H), 7.43 (dd, J = 0.9, 8.1 Hz, 1H), 7.53 (d, J = 15.2 Hz, 1H), 7.66 (d, J = 0.9 Hz, 1H), 7.98 (br s, 1H). Anal. Calcd

for $C_{22}H_{27}ClF_3N_3O_4S$: C, 50.62; H, 5.21; N, 8.05. Found: C, 50.81; H, 5.42; N, 8.19.

6.1.1.60. (E)-3-[1-[2-Chloro-4-(trifluoromethyl)benzyl]-3-(2-methoxyethoxy)-1*H*-pyrazol-5-yl]-N-[(3-me thylbutyl)sulfonyl] propenamide (30d). (*E*)-3-[1-[2-Chloro-4-(trifluoromethyl) benzyl]-3-(2-methoxyethoxy)-1*H*-pyrazol-5-yl]propenoic acid was prepared in 70% yield by a method similar to that described for **2**. Mp 169–172 °C (THF/hexane). ^{1}H NMR ($CDCl_3$): δ 3.44 (s, 3H), 3.66–3.81 (m, 2H), 4.22–4.40 (m, 2H), 5.41 (s, 2H), 6.13 (s, 1H), 6.29 (d, J = 15.8 Hz, 1H), 6.77 (d, J = 8.1 Hz, 1H), 7.36–7.52 (m, 2H), 7.66 (d, J = 0.9 Hz, 1H). Anal. Calcd for $C_{17}H_{16}ClF_3N_2O_4$: C, 50.44; H, 3.98; N, 6.92. Found: C, 50.38; H, 3.89; N, 6.85. Compound **30d** was prepared from the corresponding carboxylic acid in 54% yield by a method similar to that described for **25c**. Mp 114–116 °C (MeOH/water). ^{1}H NMR ($CDCl_3$): δ 0.91 (d, J = 6.4 Hz, 6H), 1.63–1.77 (m, 3H), 3.44 (s, 3H), 3.47 (dd, J = 2.2, 5.9 Hz, 2H), 3.60–3.84 (m, 2H), 4.20–4.42 (m, 2H), 5.40 (s, 2H), 6.13 (s, 1H), 6.30 (d, J = 15.3 Hz, 1H), 6.73 (d, J = 8.1 Hz, 1H), 7.41 (dd, J = 1.0, 8.1 Hz, 1H), 7.51 (d, J = 15.3 Hz, 1H), 7.66 (d, J = 1.0 Hz, 1H), 8.56 (br s, 1H). Anal. Calcd for $C_{22}H_{27}ClF_3N_3O_5S$: C, 49.12; H, 5.06; N, 7.81. Found: C, 49.02; H, 4.94; N, 7.73.

6.1.1.61. Methyl 3-butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazole-5-carboxylate (31). Compound **31** was prepared in 83% yield by a method similar to that described for **17a**. ^{1}H NMR ($CDCl_3$): δ 0.96 (t, J = 7.2 Hz, 3H), 1.40–1.53 (m, 2H), 1.69–1.79 (m, 2H), 3.81 (s, 3H), 4.12 (t, J = 6.6 Hz, 2H), 5.67 (s, 2H), 6.29 (s, 1H), 6.53 (d, J = 8.7 Hz, 1H), 7.11 (dd, J = 2.1, 8.1 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H).

6.1.1.62. [3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]acetonitrile (32a). [3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]methanol was prepared from **31** in 88% yield by a method similar to that described for (1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)methanol (see Section 6.1.1.4.). ^{1}H NMR ($CDCl_3$): δ 0.95 (t, J = 7.2 Hz, 3H), 1.33–1.53 (m, 2H), 1.64–1.78 (m, 2H), 4.05–4.17 (m, 2H), 4.53 (d, J = 5.7 Hz, 2H), 5.29 (s, 2H), 5.72 (s, 1H), 6.62 (d, J = 8.4 Hz, 1H), 7.14 (dd, J = 2.1, 8.4 Hz, 1H), 7.37 (d, J = 2.1 Hz, 1H). To a solution of the obtained alcohol (1.71 g, 5.19 mmol), acetone cyanohydrin (0.7 mL, 7.66 mmol), and tributylphosphine (2.4 mL, 9.61 mmol) in THF (40 mL) was added ADDP (2.40 g, 9.51 mmol). After being stirred at room temperature for 30 min, the mixture was concentrated in vacuo and the residue was diluted with IPE. The insoluble product was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was subjected to silica gel chromatography (hexane to hexane/EtOAc 3:1) to give crude **32a** (2.03 g) as a yellow oil, which was used for the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 0.96 (t, J = 7.3 Hz, 3H), 1.37–1.54 (m, 2H), 1.65–1.83 (m, 2H), 3.60 (s, 2H), 4.05–4.18 (m, 2H), 5.20 (s, 2H), 5.83 (s, 1H), 6.72 (d, J = 8.4 Hz, 1H), 7.20 (dd, J = 2.1, 8.4 Hz, 1H), 7.41 (d, J = 2.1 Hz, 1H).

6.1.1.63. 4-[3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]butanenitrile (32b). 3-[3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]propan-1-ol was prepared from **17a** in 84% yield by a method similar to that described for (1-benzyl-3-isopropoxy-1*H*-pyrazol-5-yl)methanol (see Section 7). 6.1.1.4.). ^{1}H NMR ($CDCl_3$): δ 0.95 (t, J = 7.2 Hz, 3H), 1.35–1.86 (m, 7H), 2.45–2.59 (m, 2H), 3.55–3.70 (m, 2H), 4.02–4.14 (m, 2H), 5.17 (s, 2H), 5.56 (s, 1H), 6.55 (d, J = 8.1 Hz, 1H), 7.13 (dd, J = 2.1, 8.1 Hz, 1H), 7.37 (d, J = 2.1 Hz, 1H). Compound **32b** was prepared from the corresponding alcohol in 84% yield by a method similar to that described for **32a**. ^{1}H NMR ($CDCl_3$): δ 0.96 (t, J = 7.2 Hz, 3H), 1.39–1.54 (m, 2H), 1.68–1.80 (m, 2H), 1.84–1.97 (m, 2H), 2.34–2.42 (m, 2H), 2.58–2.67 (m, 2H), 4.10 (t, J = 6.6 Hz, 2H), 5.17 (s, 2H), 5.58 (s, 1H), 6.60 (d, J = 8.4 Hz, 1H), 7.15 (dd, J = 2.1, 8.4 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H).

6.1.1.64. 3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazole-5-carboxylic acid (33a). Compound **33a** was prepared from **31** in 68% yield by a method similar to that described for **2**. Mp 154–155 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.96 (t, J = 7.4 Hz, 3H), 1.40–1.53 (m, 2H), 1.69–1.79 (m, 2H), 4.12 (t, J = 6.6 Hz, 2H), 5.66 (s, 2H), 6.29 (s, 1H), 6.55 (d, J = 8.5 Hz, 1H), 7.12 (dd, J = 2.1, 8.5 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H). Anal. Calcd for $C_{15}H_{16}Cl_2N_2O_3$: C, 52.49; H, 4.70; N, 8.16. Found: C, 52.49; H, 4.71; N, 8.22.

6.1.1.65. [3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]acetic acid (33b). The mixture of crude **32a** (2.03 g), EtOH (25 mL), and 4 M NaOH (13 mL, 52.0 mmol) was heated under reflux with stirring for 1 h. After being cooled, the mixture was acidified with 1 M HCl, concentrated in vacuo to remove organic solvents, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was recrystallized from EtOAc/hexane to give **33b** (890 mg, 48% for 2 steps) as colorless crystals: mp 105–108 °C. ^{1}H NMR ($CDCl_3$): δ 0.95 (t, J = 7.3 Hz, 3H), 1.38–1.58 (m, 2H), 1.65–1.86 (m, 2H), 3.56 (s, 2H), 4.10 (t, J = 6.6 Hz, 2H), 5.21 (s, 2H), 5.74 (s, 1H), 6.66 (d, J = 8.4 Hz, 1H), 7.15 (dd, J = 2.1, 8.4 Hz, 1H), 7.37 (d, J = 2.1 Hz, 1H). Anal. Calcd for $C_{16}H_{18}Cl_2N_2O_3$: C, 53.79; H, 5.08; N, 7.84. Found: C, 53.78; H, 5.05; N, 7.88.

6.1.1.66. 4-[3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]butanoic acid (33c). Compound **33c** was prepared from **32b** in 82% yield by a method similar to that described for **33b**. Mp 79–81 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.96 (t, J = 7.2 Hz, 3H), 1.39–1.54 (m, 2H), 1.67–1.80 (m, 2H), 1.82–1.94 (m, 2H), 2.33–2.42 (m, 2H), 2.46–2.55 (m, 2H), 4.09 (t, J = 6.6 Hz, 2H), 5.17 (s, 2H), 5.57 (s, 1H), 6.56 (d, J = 8.4 Hz, 1H), 7.14 (dd, J = 2.1, 8.4 Hz, 1H), 7.37 (d, J = 2.1 Hz, 1H). Anal. Calcd for $C_{18}H_{22}Cl_2N_2O_3$: C, 56.11; H, 5.76; N, 7.27. Found: C, 56.35; H, 5.77; N, 7.22.

6.1.1.67. 5-[3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]pentanoic acid (33d). To a 1 M BH_3·THF in THF (8.0 mL, 8.0 mmol) was added **33c** (300 mg, 0.779 mmol) portionwise. After being stirred at room temperature for 4 h, the mixture was poured into 1 M HCl and extracted with EtOAc. The extract was washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc, 19:1 to 7:3) to give 4-[3-butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]butan-1-ol (150 mg, 52%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.95 (t, J = 7.2 Hz, 3H), 1.37–1.96 (m, 8H), 2.39–2.52 (m, 2H), 3.54–3.62 (m, 2H), 4.10 (t, J = 6.9 Hz, 2H), 5.16 (s, 2H), 5.56 (s, 1H), 6.58 (d, J = 8.4 Hz, 1H), 7.14 (dd, J = 2.1, 8.4 Hz, 1H), 7.37 (d, J = 2.1 Hz, 1H). 5-[3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]pentanenitrile was prepared from the obtained alcohol in 68% yield by a method similar to that described for **32a**. ^{1}H NMR ($CDCl_3$): δ 0.96 (t, J = 7.2 Hz, 3H), 1.39–1.54 (m, 2H), 1.68–1.80 (m, 2H), 1.84–1.97 (m, 2H), 2.34–2.42 (m, 2H), 2.58–2.67 (m, 2H), 4.10 (t, J = 6.6 Hz, 2H), 5.17 (s, 2H), 5.58 (s, 1H), 6.60 (d, J = 8.4 Hz, 1H), 7.15 (dd, J = 2.1, 8.4 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H). Compound **33d** was prepared in 93% yield by a method similar to that described for **33b**. Mp 79–81 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.95 (t, J = 7.4 Hz, 3H), 1.35–1.85 (m, 8H), 2.32 (t, J = 6.9 Hz, 2H), 2.44 (t, J = 7.1 Hz, 2H), 4.10 (q, J = 6.7 Hz, 2H), 5.15 (s, 2H), 5.55 (s, 1H), 6.56 (d, J = 8.5 Hz, 1H), 7.14 (dd, J = 2.1, 8.5 Hz, 1H), 7.37 (d, J = 2.1 Hz, 1H).

6.1.1.68. 3-Butoxy-1-(2,4-dichlorobenzyl)-*N*-(pentylsulfonyl)-1*H*-pyrazole-5-carboxamide (34a). Compound **34a** was prepared from **33a** in 4% yield by a method similar to that described for **3c**.

Mp 139–141 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.82–1.03 (m, 6H), 1.22–1.53 (m, 6H), 1.66–2.01 (m, 4H), 3.36–3.65 (m, 2H), 4.13 (t, J = 6.6 Hz, 2H), 5.65 (s, 2H), 6.15 (s, 1H), 6.61 (d, J = 8.4 Hz, 1H), 7.14 (dd, J = 2.1, 8.4 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H), 8.38 (s, 1H). Anal. Calcd for $C_{20}H_{27}Cl_2N_3O_4S$: C, 50.42; H, 5.71; N, 8.82. Found: C, 50.51; H, 5.84; N, 8.90.

6.1.1.69. 2-[3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]-*N*-(pentylsulfonyl)acetamide (34b). Compound **34b** was prepared from **33b** in 25% yield by a method similar to that described for **3c**. ^{1}H NMR ($CDCl_3$): δ 0.81–1.08 (m, 6H), 1.23–1.90 (m, 10H), 3.25–3.36 (m, 2H), 3.60 (s, 2H), 4.11 (t, J = 6.6 Hz, 2H), 5.20 (s, 2H), 5.74 (s, 1H), 6.75 (d, J = 8.4 Hz, 1H), 7.19 (dd, J = 2.1, 8.4 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H).

6.1.1.70. 4-[3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]-*N*-(pentylsulfonyl)butanamide (34c). Compound **34c** was prepared from **33c** in 54% yield by a method similar to that described for **3c**. Mp 121–122 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.85–0.99 (m, 6H), 1.27–1.54 (m, 6H), 1.66–1.85 (m, 4H), 1.86–2.02 (m, 2H), 2.34 (t, J = 7.1 Hz, 2H), 2.51 (t, J = 7.5 Hz, 2H), 3.34–3.45 (m, 2H), 4.08 (t, J = 6.6 Hz, 2H), 5.15 (s, 2H), 5.55 (s, 1H), 6.56 (d, J = 8.4 Hz, 1H), 7.14 (dd, J = 2.1, 8.4 Hz, 1H), 7.37 (d, J = 2.1 Hz, 1H), 7.93 (br s, 1H). Anal. Calcd for $C_{23}H_{33}Cl_2N_3O_4S$: C, 53.28; H, 6.42; N, 8.10. Found: C, 53.34; H, 6.45; N, 8.11.

6.1.1.71. 5-[3-Butoxy-1-(2,4-dichlorobenzyl)-1*H*-pyrazol-5-yl]-*N*-(pentylsulfonyl)pentanamide (34d). Compound **34d** was prepared from **33d** in 50% yield by a method similar to that described for **3c**. Mp 91–93 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$): δ 0.84–1.01 (m, 6H), 1.23–1.90 (m, 14H), 2.29 (t, J = 7.1 Hz, 2H), 2.45 (t, J = 7.2 Hz, 2H), 3.34–3.46 (m, 2H), 4.09 (t, J = 6.6 Hz, 2H), 5.15 (s, 2H), 5.55 (s, 1H), 6.58 (d, J = 8.3 Hz, 1H), 7.15 (dd, J = 2.1, 8.3 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H), 7.90 (br s, 1H). Anal. Calcd for $C_{24}H_{35}Cl_2N_3O_4S$: C, 54.13; H, 6.62; N, 7.89. Found: C, 54.10; H, 6.54; N, 7.90.

6.1.2. Metabolic stability assay

Human or rat hepatic microsomes were purchased from Xenotech, LLC (Lenexa, KS). An incubation mixture with a final volume of 0.1 mL consisted of microsomal protein in 50 mmol/L phosphate buffer (pH 7.4) and 1 μmol/L test compound. The concentration of hepatic microsomal protein was 0.2 mg/mL. An NADPH-generating system containing 50 mmol/L $MgCl_2$, 50 mmol/L glucose-6-phosphate, 5 mmol/L beta-$NADP^+$ and 15 unit/mL glucose-6-phosphate dehydrogenase was prepared and added to the incubation mixture with a 10% volume of the reaction mixture. After the addition of the NADPH-generating system, the mixture was incubated at 37 °C for 0 and 20 min. The reaction was terminated by the addition of equivalent volume of MeCN to that of the reaction mixture. All incubations were made in duplicate. The amounts of test compound in the reaction mixtures were measured by HPLC analysis which was performed by Nemoto Science Co., Ltd (Tokyo, Japan). Metabolic stability was assessed as disappearance rate of the parent compound. Disappearance rate is represented as a percentage of the amount of the parent compound in the reaction mixture for 20 min relative to that in the reaction mixture for 0 min taken as 100%.

6.1.3. Pharmacokinetic analyses in rat cassette

Dosing test compounds were administered as a cassette dosing to nonfasted rats. After oral and intravenous administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with MeCN containing an internal standard. After centrifugation, the supernatant was diluted and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

6.1.4. Biology

Establishment of a stable transformed cell expressing human PPARγ1, human RXRα, and PPAR responsive luciferase repoter gene. The full length human PPARγ1, full length human RXRα, and PPAR responsive luciferase reporter gene were stably expressed in CHO-K1 cells. Transfection of mammalian expression plasmid, pVGRXR2-hPPARγ-zeo, and reporter plasmid, pGL3-(PPREx4)-Tk-neo, into the cells was performed by electroporation using a Gene Pulser (Bio-Rad, Japan). G418 (Life Technologies Inc., U.S.A.) and Zeocin (Invitrogen, U.S.A.) resistant clones were selected and examined for their ability to introduce liciferase expression in the presence of pioglitazone. Finally, we selected the clone, PPARγ:RXRα:4ERPP/CHO-K1 No. 10, which expressed high levels of luciferase activity by pioglitazone.

6.1.5. Transactivation assay of hPPARγ1

PPARγ:RXRα:PPRE×4/CHO-K1 cells were used for transactivation assays of hPPARγ1. These cells were seeded into an OPAQUE PLATE (white 96 well half area plate, COSTAR, U.S.A.) at the density of 1×10^4 cells/well, and cultured in 5% CO_2 at 37 °C overnight. After washing the OPAQUE PLATE with PBS, 45 μL of HAM F12 medium containing 0.1% fatty acid free-BSA and 5 μL of test compounds were added to the plate, which was then cultured in 5% CO_2 at 37 °C for 1 day. After removing the medium, 20 μL of PICAGENE-LT7.5 (Wako Pure Chemical Ind., Ltd, Japan), which was diluted to half with HANK'S BALANCED SALT SOLUTION, was added to each well. After stirring, luciferase activities were determined in microplate-based luminescence reader (Perkinnelmer, U.S.A.)

Transient co-transfection assay of hPPARα and hPPARδ. COS-1 cells were seeded at 5×10^6 cells in 150 cm^2 tissue culture flask, and cultured in 5% CO_2 at 37 °C overnight. Transfections were performed with LipofectAMINE (Life Technologies, Inc., U.S.A.) according to the instructions of manufacturer. Briefly, the transfection mixture contained 125 μL of LipofectAMINE, 100 μL of plus reagent, 2.5 μg of each expression plasmid pMCMVneo-hPPARα (pMCMVneo-hPPARδ) and pMCMVneo-hRXRα, 5 μg of reporter plasmid pGL3-PPRE×4-tk-luc-neo, and 5 μg of pRL-tk (Promega, U.S.A.). Cells were incubated in 25 mL of transfection mixture for 3 h in 5% CO_2 at 37 °C. After adding 25 mL of DMEM medium (Life Technologies, Inc., U.S.A.) containing 0.1% fatty acid free-BSA, the cells were then incubated for 1 day in 5% CO_2 at 37 °C. After transfection, cells were detached by treating with trypsin-EDTA (Life Technologies INC., U.S.A.) centrifuged and then suspended in DMEM medium containing 0.1% fatty acid free-BSA. The suspended cells were added into an OPAQUE PLATE (white 96 well half area plate, COSTAR, U.S.A.) at the density of 5×10^3 cells/well in 45 μL of DMEM medium containing 0.1% fatty acid free-BSA and 5 μL of test compounds and then cultured in 5% CO_2 at 37 °C for 2 days. After removing the medium, 20 μL of PICAGENE-LT7.5 (Wako Pure Chemical Ind., Ltd, Japan), which was diluted to half with HANK'S BALANCED SALT SOLUTION, was added to each well. After stirring, luciferase activities were determined in microplate-based luminescence reader (Perkinnelmer, U.S.A.).

6.1.6. Evaluation of plasma glucose- and triglyceride-lowering activities in Wistar fatty rats

Male Wistar fatty rats were obtained from Takeda Rabics (Japan) and were used in these experiments. Throughout the study, they were housed in metal cages and fed a commercial diet CE-2 (Clea, Japan) and water ad libitum. At the beginning of experiment, blood samples were withdrawn from tail vein with heparin as anticoagulant, and initial body weight was measured. Plasma samples were obtained from blood samples by centrifugation. Plasma levels

of glucose and triglyceride were measured enzymatically using Autoanalyzer 7080 (Hitachi, Japan). Rats were divided based on body weight, plasma glucose and triglyceride (n = 5/group). Wistar fatty rats were orally administered test compounds suspended in 0.5% methylcellurose solution once daily for 7 days. The next day of final administration, blood samples were withdrawn from tail vein, and body weight was measured. After obtaining of plasma samples by centrifugation of blood samples, we measured plasma glucose and triglyceride. The change (%) from the initial levels of plasma glucose and triglyceride after 7-day treatment was calculated in each group. The degree of the lowering effect in each group was estimated from the relative ratio of the change (%) in each group to the change in the control group. Then, the ED_{25} for the glucose- and triglyceride-lowering effects of test compounds were calculated from the dose-response curves of the relative ratios generated by logistic regression using the PCP systems.

6.1.7. Docking study

The atomic coordinates of the crystal structures (1FM6 and 1PRG) were obtained from the web site of RCSB protein data bank (http://www.rcsb.org/pdb/home/home.do). Both protein structures were added hydrogens and then only hydrogens were energetically optimized in MOE (ver. 2000.02, Chemical Computing Group Inc, Canada.). Each compound was docked into the PPARγ-LBD using the GOLD program (ver. 2.0, the Cambridge Crystallographic Data Centre, UK) with the default parameter set. After some manual adjustment to remove large steric hindrances, docking poses showing high score (Gold score) were subjected to energy minimization using MMFF94s force field in MOE. During the energy minimization procedure firstly whole protein structure was fixed and secondly that within 3.5 Å from the each ligand was relaxed.

6.1.8. X-ray co-crystallography study. Protein expression and purification

The ligand binding domain (LBD) of human PPARγ (NM_005037; aa 202-475) was cloned into pET28a vector (Novagen) and expressed as an N-terminal 6xHis fusion protein in *Escherichia coli*. Fusion protein was purified by Ni affinity chromatography (Probond, Invitrogen) at pH 7.9, followed by anion exchange (Q Sepharose FF, GE Healthcare) and size exclusion chromatography (Superdex S200, GE Healthcare). Before crystallization, the protein solution was concentrated to 16 mg/ml by centrifugal ultrafiltration.

6.1.9. Crystallization and data collection

Crystals of the PPARγ-**3d** complex were obtained by pre-incubating the 16 mg/mL protein preparation with 2.0 mM compound, and were grown at room temperature by sitting-drop vapor diffusion using Nanovolume Crystallization techniques with 50 nL of protein solution and 50 nL of reservoir containing 2.8 M sodium acetate, pH 7.0. Single crystals were harvested in reservoir solutions supplemented with 25% glycerol as cryoprotectant and flash-frozen by direct immersion in liquid nitrogen. X-ray diffraction data were collected at the Advanced Light Source in Berkeley, California at Beam Line 5.0.3 equipped with an ADSC Quantum 4r CCD detector. The diffraction intensities were integrated and scaled using the HKL2000 program suite.[22] Data collection statistics are presented in Table 7.

6.1.10. Structure determination and refinement

The structure was determined by molecular replacement using MolRep[23] and 2PRG[7a] as the search model. The initial solutions were refined with REFMAC,[24] and the models were visually inspected and manually built and rebuilt using the XtalView/Xfit program suite. [25] The crystallographic refinement statistics are presented in Table 7.

Table 7
X-ray data collection and refinement statistics

	3d
Crystal	
Space group	$P4_32_12$
Unit cell dimensions	a = 65.5 Å
	b = 65.5 Å
	c = 154.2 Å
Molecules/asymmetric unit	1
Data collection	
Resolution [Å] (outer shell)	50−1.90 (1.97−1.90)
Observations (unique)	279699 (26985)
Redundancy	10.4
Completeness overall (outer shell)	98.3 (94.1)%
I/σ (I) overall (outer shell)	33.3 (5.1)
R_{symm} overall (outer shell)	0.059 (0.416)
Refinement	
Resolution [Å]	20–1.9
Reflections used	25478
R-factor	0.187
R_{free}	0.219
RMS bonds [Å]	0.006
RMS angles [°]	1.10
Average B-value [Å^2]	24.8

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