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Synthesis and structure–activity relationships of thiadiazole-derivatives as potent and orally active peroxisome proliferator-activated receptors α/δ dual agonists

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Abstract—Replacement of the methyl-thiazole moiety of GW501516 (a PPARδ selective agonist) with [1,2,4]thiadiazole gave compound **21** which unexpectedly displayed submicromolar potency as a partial agonist at PPARα in addition to the high potency at PPARδ. A structure–activity relationships study of **21** resulted in the identification of **40** as a potent and selective PPARα/δ dual agonist. Compound **40** and its close analogs represent a new series of PPARα/δ dual agonists. The high potency, high selectivity, significant gene induction, excellent PK profiles, low P450 inhibition or induction, and good in vivo efficacy in four animal models support **40** being selected as a pre-clinical study candidate, and may render **40** as a valuable pharmacological tool in elucidating the complex roles of PPARα/δ dual agonists, and the potential usage for the treatment of metabolic syndrome.
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1. Introduction

Cardiovascular disease (CVD) is the most common cause of morbidity and mortality in developed nations.[1] Atherogenic dyslipidemia, characterized by an abnormal circulating lipid profile including low levels of high density lipoprotein cholesterol (HDL-C), elevated levels of small-dense low density lipoprotein cholesterol (LDL-C) or elevated triglycerides (TG), is often found in patients who are obese, have type 2 diabetes or the metabolic syndrome.[2–4] These individuals are at high risk for premature CVD. While LDL-C is prone to accumulate in the arterial wall leading to the formation of atherosclerotic cholesterol-laden foam cells,[5] HDL-C may play a protective role in removing excess cholesterol from peripheral cells and return it to the liver.[6]

The peroxisome proliferator-activated receptors (PPARs) belong to the nuclear hormone receptor superfamily and consist of three members, PPARα, PPARγ, and PPARδ. PPARα is expressed mainly in tissues involved in lipid oxidation such as liver, kidney, adrenal glands, cardiac muscle, and skeletal muscles. PPARα regulates the expression of genes involved in lipid metabolism.[7] The fibrate drugs, such as fenofibrate **90** and ciprofibrate **91** (Fig. 1), have been used for the clinical treatment of dyslipidemia by lowering serum triglycerides, free fatty acid (FFA) levels and raising HDL-C since the 1970s.[8] Several studies have provided evidence that the hypolipidemic effect of the fibrate drugs is attributed to the activation of PPARα.[9] PPARγ is expressed in adipose tissue, macrophages, and vascular smooth muscles.[7] PPARγ was identified as a key regulator for insulin sensitivity, and two PPARγ agonists, rosiglitazone **92** and pioglitazone **93**, have been used for the clinical treatment of Type 2 diabetes since 1999.[10] In contrast, the biological role of PPARδ and the potential clinical utility of its ligand was unclear, in part, due to its broad tissue expression[11] and lack of good chemical tools to study its pharmacology. Recently, a potent

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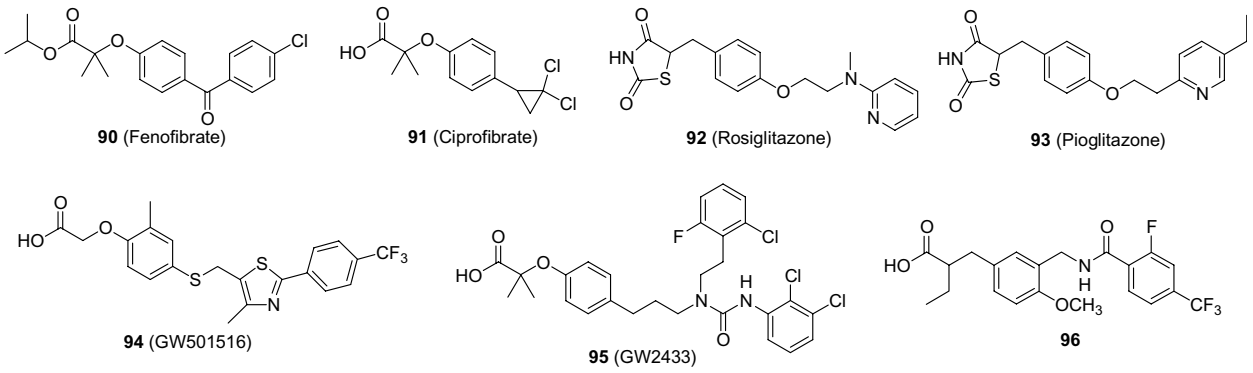
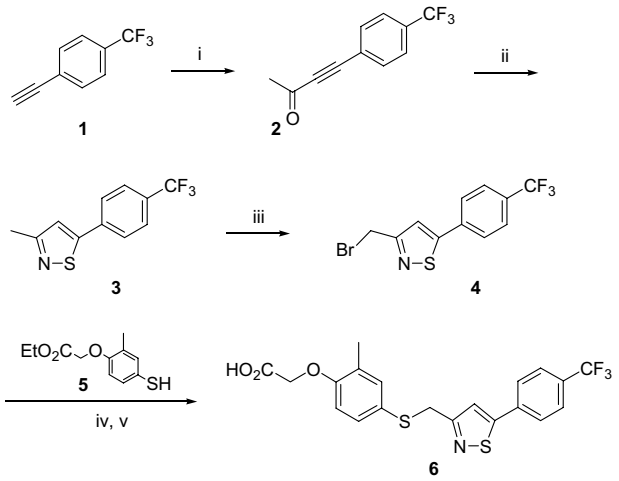


Figure 1. Structures of representative PPAR agonists: PPARα (**90**, **91**), PPARγ (**92**, **93**), PPARδ (**94**), and PPARα/δ (**95**, **96**).

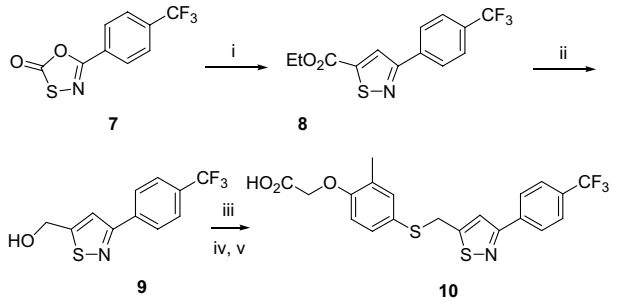
and selective PPARδ agonist **94** (GW501516, phase-II clinical trial) was developed and shown to increase plasma HDL-C levels together with decreasing LDL-C and triglycerides in obese and dyslipidemic rhesus monkeys.[12] In addition, while one study supported an atheroprotective effect of PPARδ agonists in LDLR−/− mice,[13] other data suggested that PPARδ activation may attenuate the metabolic syndrome including obesity.[14] To effectively target dyslipidemia to reduce the risk of cardiovascular diseases, it may be beneficial to activate PPARα and PPARδ simultaneously through a single molecule. To date, there are only two series of PPARα/δ dual agonists (**95** (GW2433)[15] and **96**[16]) with in vitro data reported. A preliminary report[17] from our laboratory described the finding of [1,2,4]thiadiazole-derivatives as a new series of potent and orally active PPARα/δ dual agonists, however, the best compound was found to have P450 induction issue later. This article describes a full report of the SAR study and identification of a potent and orally active PPARα/δ dual agonist that lacks P450 induction, therefore, being selected as a pre-clinical study candidate.

2. Chemistry

The synthesis of the first target molecule **6** is shown in Scheme 1. Trapping the anion of alkyne **1** with acetic anhydride at 20–35 °C gave **2**. Cyclocondensation of **2** with hydroxylamine-*O*-sulfonic acid at 20 °C provided the isothiazole **3**. Bromination of **3** with NBS and benzoyl peroxide in refluxing CCl_4 gave the bromide **4**. S-alkylation of **4** with thiophenoxide **5**, followed by base hydrolysis of the ethyl ester, provided the desired target molecule **6**. The synthesis of the isothiazole **10** is shown in Scheme 2. Treatment of [1,3,4]-oxathiazol-2-one **7**[17] with ethyl propiolate at 160 °C with the extrusion of CO_2 gave isothiazole-5-carboxylic acid ethyl ester **8** and isothiazole-4-carboxylic acid ethyl ester **11** in almost 1:1 ratio. Reduction of the ethyl ester **8** with LAH resulted in the formation of primary alcohol **9**. Conversion of **9** to its mesylate, followed by S-alkylation with **5** and base hydrolysis of the ethyl ester, gave the target **10**. In the meanwhile, reduction of the ethyl ester **11** (Scheme 3) with $NaBH_4$ at 20 °C yielded the primary alcohol **12**. Conversion of **12** to its mesylate, followed by S-alkylation and base hydrolysis, provided the target

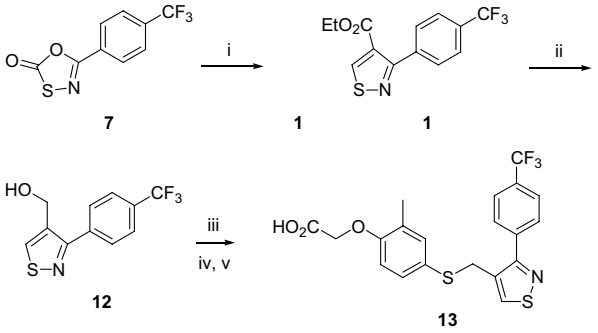


Scheme 1. Reagents and conditions: (i) EtMgBr, Ac_2O, THF, 20–35 °C, 94%; (ii) H_2NOSO_3H, NaSH, H_2O, 70%; (iii) $(PhCO)_2O_2$, NBS, CCl_4, reflux, 42%; (iv) 5, CH_3CN, Cs_2CO_3; (v) NaOH, MeOH−H_2O, 75% (two steps).

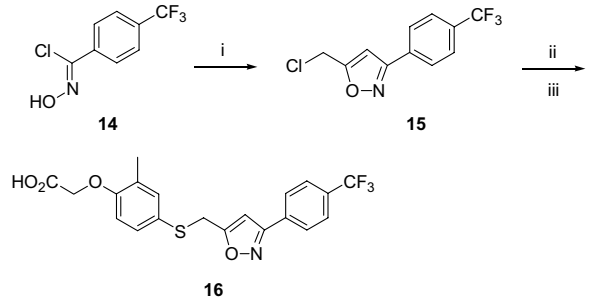


Scheme 2. Reagents and conditions: (i) ethyl propiolate, 1,2-dichlorobenzene, 160 °C, 31%; (ii) LAH, THF; (iii) MsCl, Et_3N, CH_2Cl_2; (iv) 5, CH_3CN, Cs_2CO_3; (v) NaOH, MeOH−H_2O, 39% (four steps).

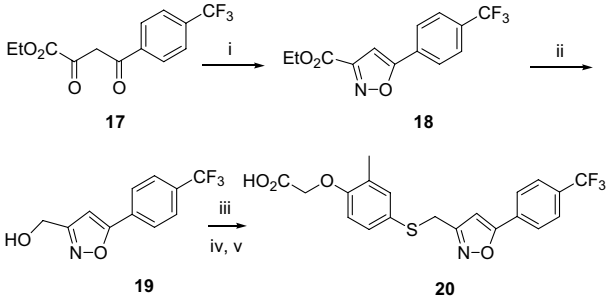
13. The synthesis of the isoxazole **16** is shown in Scheme 4. Cyclocondensation of chloro-oxime **14**[18] with propargyl chloride at 20 °C gave isoxazole **15**. S-alkylation of **15** with **5**, followed by base hydrolysis, provided the target **16**. The synthesis of reverse-isoxazole **20** is shown in Scheme 5. Cyclocondensation of 2,4-dioxo-ester **17**[19] with hydroxylamine at 78 °C yielded isoxazole-3-carboxylic acid ethyl ester **18**. Reduction of **18** with LAH at −78 °C gave the primary alcohol **19**. Conversion of



Scheme 3. Reagents and conditions: (i) ethyl propiolate, 1,2-dichlorobenzene, 160 °C, 36%; (ii) $NaBH_4$, EtOH, 93%; (iii) MsCl, Et_3N, CH_2Cl_2; (iv) 5, CH_3CN, Cs_2CO_3; (v) NaOH, MeOH–H_2O, 72% (three steps).

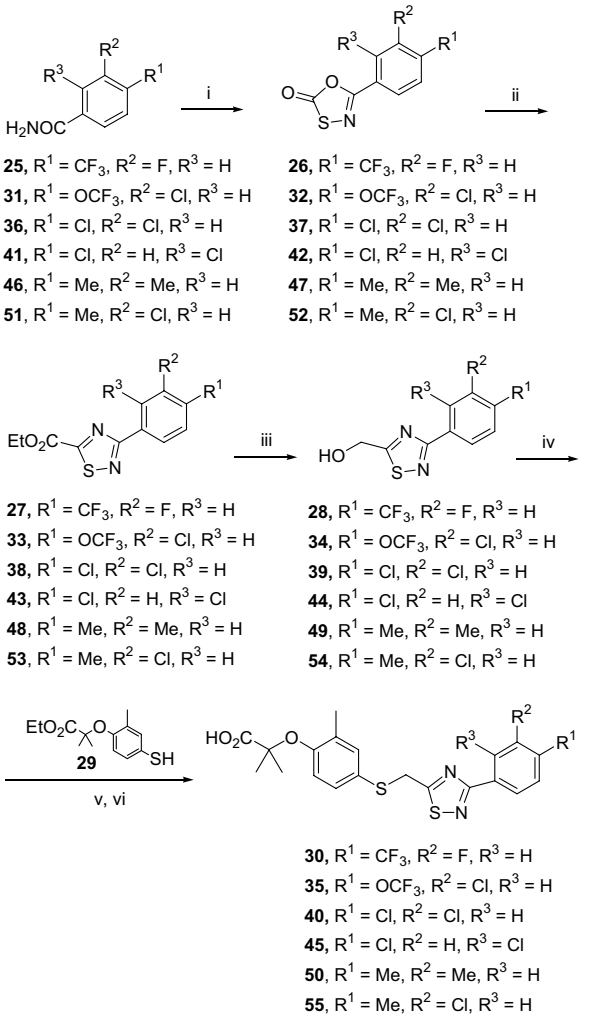


Scheme 4. Reagents and conditions: (i) propargyl chloride, CH_2Cl_2, Et_3N, 64%; (ii) 5, CH_3CN, Cs_2CO_3; (iii) NaOH, MeOH–H_2O, 91% (two steps).

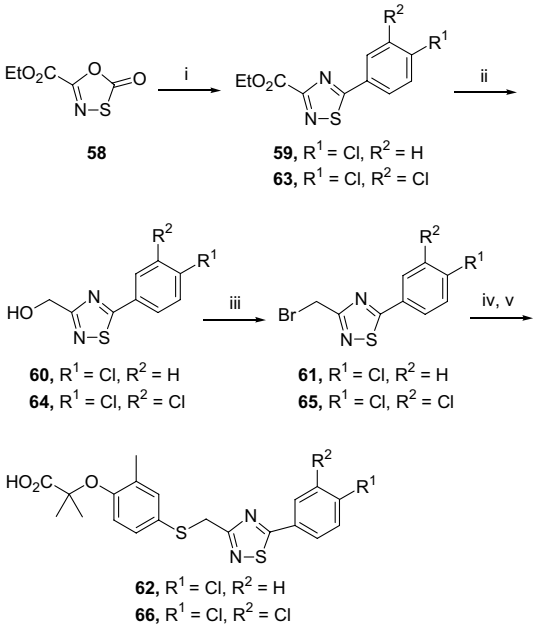


Scheme 5. Reagents and conditions: (i) NH_2OH, HCl, EtOH, 78 °C, 91%; (ii) LAH, THF, −78 °C, 45%; (iii) MsCl, Et_3N, CH_2Cl_2; (iv) 5, CH_3CN, Cs_2CO_3; (v) NaOH, MeOH–H_2O, 78% (three steps).

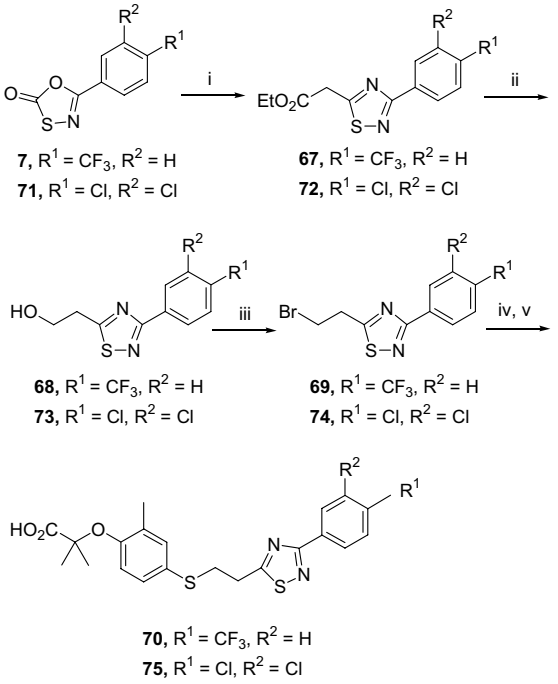
19 to its mesylate, followed by S-alkylation with **5**, and base hydrolysis of the ester, provided isoxazole target **20**. The synthesis of [1,2,4]thiadiazole **30** is shown in Scheme 6. Reaction of substituted benzamide **25** with chlorocarbonylsulfenyl chloride at 60 °C yielded [1,3,4]oxathiazol-2-one **26**. Cyclocondensation of **26** with ethyl cyanoformate at 160 °C gave [1,2,4]thiadiazole-5-carboxylic acid ethyl ester **27**. Reduction of **27** with $NaBH_4$ provided primary alcohol **28**. Conversion of **28** to the bromide, S-alkylation of the bromide with **29**,[17] followed by base hydrolysis, gave the desired target **30**. The analogs **35**, **40**, **45**, **50**, and **55** were all prepared by the same method as that of **30**. The synthesis of reverse-thiadiazole **62** is shown in Scheme 7. Cyclocondensation of **58** with 4-chloro-benzonitrile at 160 °C with the extrusion of CO_2 gave [1,2,4]thiadiazole-3-carboxylic acid ethyl ester **59**. Reduction of **59** to its primary alcohol **60**, followed by the conversion of **60** to the bromide **61**, S-alkylation with **29**, and base hydrolysis, yielded the desired target **62**. Analog **66** was prepared in the same route. The synthesis of two-methylene-link thiadiazole analog **70** is shown in Scheme 8. Cyclocondensation of **7** with ethyl cyanoacetate at 160 °C provided thiadiazole **67**. Reduction of **67** with $NaBH_4$ gave the alcohol **68**, bromination of **68** yielded the bromide **69**, S-alkylation of **69**, followed by base hydrolysis, gave target **70**. Analog **75** was prepared in the same route. The synthesis of oxygen-link thiadiazole **79** is shown in Scheme 9. Conversion of the primary alcohol **28** to its bromide, followed by O-alkylation with **78**, and base hydrolysis, provided the target **79**. The analogs **80**–**83** were all prepared in the same route. The synthesis of des-methyl thiadiazole **86** is shown in Scheme 10. Conversion of the alcohol **84**[17] to its mesylate, followed by S-alkylation with 4-mercaptophenol, gave phenol **85**. O-alkylation of **85** with *t*-$BuO_2C(CH_3)_2CBr$ at 70 °C



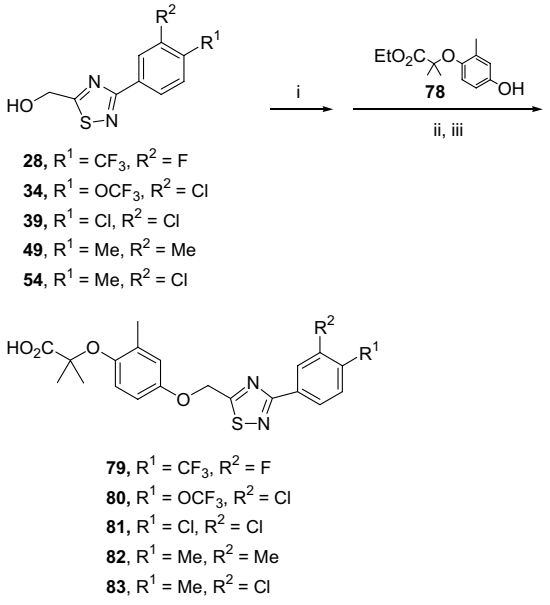
Scheme 6. Reagents and conditions: (i) chlorocarbonylsulfenyl chloride, toluene, 60 °C, 47–96%; (ii) ethyl cyanoformate, 1,2-dichlorobenzene, 160 °C, 36–91%; (iii) $NaBH_4$, EtOH, 56–93%; (iv) PPh_3, CBr_4, CH_2Cl_2; (v) 29, CH_3CN, Cs_2CO_3; (vi) NaOH, MeOH–H_2O, 20–67% (three steps).



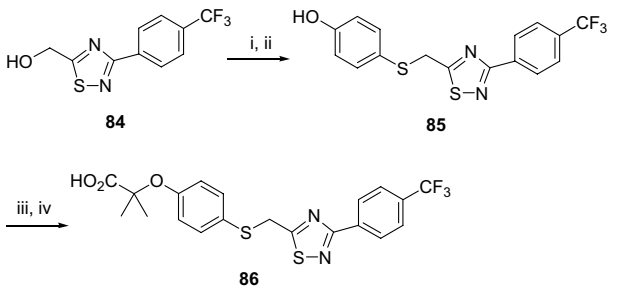
Scheme 7. Reagents and conditions: (i) R^1R^2-benzonitrile, 1,2-dichlorobenzene, 160 °C, 13–31%; (ii) $NaBH_4$, EtOH, 84–97%; (iii) PPh_3, CBr_4, CH_2Cl_2, 0–20 °C, 83–84%; (iv) 29, CH_3CN, Cs_2CO_3; (v) NaOH, MeOH–H_2O, 46–68% (two steps).



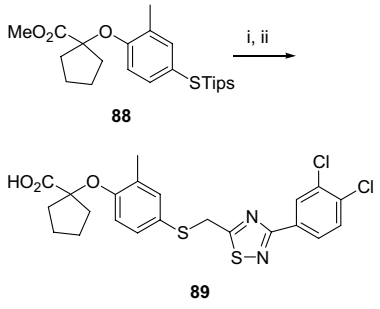
Scheme 8. Reagents and conditions: (i) ethyl cyanoacetate, 1,2-dichlorobenzene, 160 °C, 12–14%; (ii) $NaBH_4$, EtOH, 64%; (iii) PPh_3, CBr_4, CH_2Cl_2, 0–20 °C, 44–86%; (iv) 29, CH_3CN, Cs_2CO_3; (v) NaOH, MeOH–H_2O, 35–40% (two steps).



Scheme 9. Reagents: (i) PPh_3, CBr_4, CH_2Cl_2; (ii) 78, CH_3CN, Cs_2CO_3; (iii) NaOH, MeOH–H_2O, 15–51% (three steps).



Scheme 10. Reagents and conditions: (i) MsCl, Et_3N, CH_2Cl_2; (ii) 4-mercapto-phenol, CH_3CN, Cs_2CO_3, 83% (two steps); (iii) NaH, *t*-$BuO_2C(CH_3)_2CBr$, THF, 70 °C; (iv) TFA, CH_2Cl_2, 15% (two steps).



Scheme 11. Reagents and conditions: (i) 39A, TBAF, THF, 0 °C, 93%; (ii) NaOH, MeOH–H_2O, 35%.

provided **86**-*tert*-butyl ester, that was deprotected under acidic condition to give the target **86**. The synthesis of cyclopentyl-analog **89** is shown in Scheme 11. Treatment of silyl-protected thiophenol **88**[17] with the bromide of **39** at 0 °C in the presence of TBAF gave **89**-methyl ester. Base hydrolysis of the ester provided the target **89**.

3. Results and discussion

3.1. Lead generation

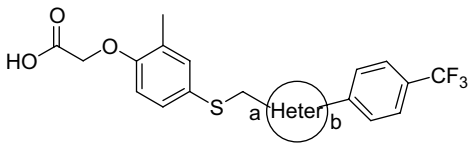
During this study, HEK293 cells were grown in DMEM/F-12 medium supplemented with 10% FBS and glutamine. The cells were co-transfected with DNA constructs containing the ligand-binding domains

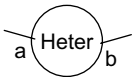
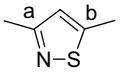
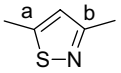
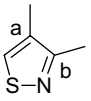
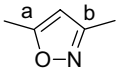
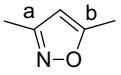
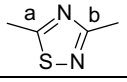
of either PPARα, γ or δ-Gal4 chimeric receptors. The steady-Glo luciferase assay kit was used for measuring luciferase reporter activity. Percent efficacies for PPARα, PPARδ, and PPARγ activation were reported relative to PPARα agonist ((*S*)-2-{2-[1-ethyl-3-(4-trifluoro-methoxyphenyl)ureido]indan-5-ylsulfanyl}-2-methylpropionic acid, compound **2.1**),[20] PPARδ agonist ({4-[2-methoxy-3-(4-trifluoromethyl-phenoxy)-propylsulfanyl]-2-methyl-phenoxy}-acetic acid, compound **2**),[21] and PPARγ agonist (rosiglitazone), respectively.

A primary goal of our PPAR research programs has been the identification of a functionally potent and selective PPARδ agonist with optimal in vivo efficacy, and maximum safety margin. Since **5** is the first reported potent and selective PPARδ agonist, we were interested in conducting structure–activity relationship (SAR) studies with respect to **5** as the lead molecule. It seems the methyl-substituted thiazole of **5** may contribute uniquely to its high functional potency and selectivity at PPARδ, therefore, we first screened various heteroaryls or heterocycles in human PPAR co-transfection assays to see if we could uncover any new series that may meet our program goal. Replacement of the methylthiazole of **5** with isothiazole gave compound **6**. As compared to **5** (EC_{50} = 1 nM at PPARδ and EC_{50} = 1100 nM at PPARα),[22] **6** displayed very similar potency at PPARδ (EC_{50} = 2 nM, 96%, Table 1) but much weaker potency at PPARα (EC_{50} > 3000 nM). It seems **6** represents another potent and selective PPARδ agonist series. Meanwhile, we also synthesized the reverse-isothiazole **10**. With the interchanged positions of nitrogen and sulfur in the ring, compound **10** exhibited slightly lower potency at PPARδ (EC_{50} = 5 nM, 94%) and lower selectivity against PPARα (EC_{50} = 1290 nM, 43%) compared to **6**. Changing the substitution pattern on the isothiazole ring from [3,5]- (as in **10**) to [3,4]-positions gave **13**. The potency of **13** displayed at PPARδ was decreased significantly (EC_{50} = 150 nM). Therefore, to enhance PPARδ potency, the polar carboxyl group may prefer to orient away from the hydrophobic 4-CF_3-phenyl group as much as possible. Replacement of the methylthiazole of **5** with isoxazole gave **16**. Compound **16** displayed ~4-fold weaker potency at PPARδ (EC_{50} = 20 nM) and even lower activity at PPARα (EC_{50} > 3000 nM) compared to isothiazole **10** (EC_{50} = 5 nM at PPARδ, EC_{50} = 1290 nM at PPARα). The reverse-isoxazole **20** exhibited high potency at PPARδ (EC_{50} = 2 nM, 80%) and modest potency at PPARα (EC_{50} = 2208 nM, 41%). Overall, we demonstrated that both isothiazole **6** and isoxazole **20** displayed comparable potency and selectivity at PPARδ compared to methylthiazole **5**. Continuing replacement of the methylthiazole of **5** with [1,2,4]thiadiazole gave **21**.[17] Unexpectedly, thiadiazole **21** exhibited submicromolar potency at PPARα as a partial agonist (EC_{50} = 468 nM, 42%) in addition to the good potency observed at PPARδ (EC_{50} = 10 nM, 79%).

In order to identify a dual PPARα/δ agonist with desired efficacy and safety profiles, we decided to focus the SAR study on the new [1,2,4]thiadiazole **21** series. Since geminal dimethyl substitution of the acidic head moiety appears to be beneficial for many PPARα agonists, including **1**, **2**, and **6**, we prepared the geminal dimethyl analog of **21** first. Indeed, **22** displayed ~6-fold higher potency at PPARα (EC_{50} = 79 nM, 51%, Table 2), slightly better potency at PPARδ (EC_{50} = 6 nM, 76%), and moderate potency with low activity at PPARγ (EC_{50} = 407 nM, 25%) compared to **21**. Replacing 4-CF_3 of **22** with 4-OCF_3 gave **23** with another 2-fold higher potency observed at PPARα (EC_{50} = 33 nM, 44%), 2-fold higher potency at PPARδ (EC_{50} = 3 nM, 73%) but abolished activity at PPARγ (EC_{50} > 3000 nM) compared to **22**. Replacing 4-CF_3 of **22** with 4-Cl gave **24** with 2-fold reduced potency observed at PPARα (EC_{50} = 186 nM, 38%), 3-fold reduced potency at PPARδ (EC_{50} = 18 nM, 72%), and abolished activity at PPARγ (EC_{50} > 3000 nM) compared to **22**. Interestingly, installation of a substituent at the 3-position of the phenyl ring in addition to the original 4-substituent seems to further enhance the potency at PPARα while maintaining the high potency at PPARδ. For example, adding a 3-F substituent to **22** gave 3-F,4-CF_3 analog **30**. Analog **30** displayed 2-fold higher potency at PPARα (EC_{50} = 34 nM, 53%) compared to **22** (EC_{50} = 79 nM, 51%) and maintained the equally high potency at PPARδ (EC_{50} = 5 nM, 68% for **30** versus EC_{50} = 6 nM, 76% for **22**), and similar low activity at

Table 1. Activities in human PPAR co-transfection assays[a]



Compound		EC_{50}[b] (nM)	
		PPARα[c] (% resp.)	PPARδ[d] (% resp.)
6		>3000	2 (96)
10		1290 (43)	5 (94)
13		>3000	150 (86)
16		>3000	20 (100)
20		2208 (41)	2 (80)
21		468 (42)	10 (79)

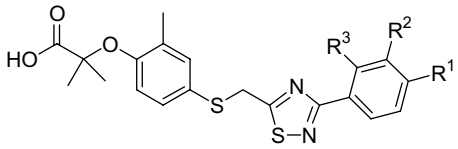
[a] All compounds displayed EC_{50} > 3000 nM at PPARγ.

[b] EC_{50} is the concentration of test compounds needed to induce 50% of the maximum luciferase activity. The EC_{50} value is the average of more than two separate tests.

[c] The internal PPARα agonist (compound **2.1**)[20] was used as the standard.

[d] The internal PPARδ agonist (compound **2**)[21] was used as the standard.

Table 2. Activities in human PPAR co-transfection assays



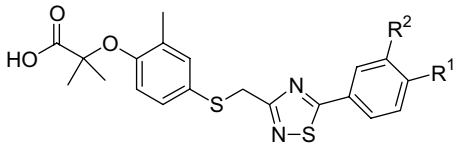
Compound	R^1	R^2	R^3	EC_{50}[a] (nM)		
				PPARα (% resp.)	PPARδ (% resp.)	PPARγ[b] (% resp.)
22	CF_3	H	H	79 (51)	6 (76)	407 (25)
23	OCF_3	H	H	33 (44)	3 (73)	>3000
24	Cl	H	H	186 (38)	18 (72)	>3000
30	CF_3	F	H	34 (53)	5 (68)	640 (22)
35	OCF_3	Cl	H	28 (73)	3 (68)	>3000
40	Cl	Cl	H	100 (56)	8 (65)	>3000
45	Cl	H	Cl	476 (33)	102 (63)	>3000
50	CH_3	CH_3	H	115 (45)	93 (66)	>3000
55	CH_3	Cl	H	70 (74)	25 (65)	669 (23)

[a] EC_{50} is the concentration of test compounds needed to induce 50% of the maximum luciferase activity. The EC_{50} value is the average of more than two separate tests.
[b] Rosiglitazone was used as the standard.

PPARγ (EC_{50} = 640 nM, 22%). Installation of a 3-Cl substituent to **23** gave 3-Cl,4-OCF_3 analog **35**. Compound **35** also exhibited higher potency and even higher activity at PPARα (EC_{50} = 28 nM, 73%) compared to **23** (EC_{50} = 33 nM, 44%), and the same potency at PPARδ (EC_{50} = 3 nM, 68% for **35** versus EC_{50} = 3 nM, 73% for **23**). Consistently, addition of the 3-Cl substituent to **24** gave 3-Cl,4-Cl analog **40** that also displayed higher potency at PPARα (EC_{50} = 100 nM, 56%) compared to **24** (EC_{50} = 186 nM, 38%), and even higher potency at PPARδ (EC_{50} = 8 nM, 65% for **40** versus EC_{50} = 18 nM, 72% for **24**). On the other hand, installation of a 2-substituent in addition to the 4-substituent of the phenyl ring reduced the potency at both PPARα and PPARδ. For example, compound **45** with 2-Cl,4-Cl substituents displayed lower potency at PPARα (EC_{50} = 476 nM, 33%) and PPARδ (EC_{50} = 102 nM, 63%) compared to **24** (EC_{50} = 186 nM, 38% at PPARα and EC_{50} = 18 nM, 72% at PPARδ). In order to expand SAR around these PPARα/δ dual agonists, we also prepared 3-CH_3,4-CH_3 analog **50** and 3-Cl,4-CH_3 analog **55**. Compound **50** displayed significant potency at PPARα (EC_{50} = 115 nM, 45%), but reduced potency at PPARδ (EC_{50} = 93 nM, 66%) compared to **30**, **35** or **40**. Compound **55** showed better potency at both PPARα (EC_{50} = 70 nM, 74%) and PPARδ (EC_{50} = 25 nM, 65%) compared to **50**, but not as selective against PPARγ (EC_{50} = 669 nM, 23%). Overall, **23**, **24**, **35**, **40**, and **50** represented interesting potent PPARα/δ dual agonists.

We next examined the reverse-[1,2,4]thiadiazole series to see if the specific orientation of the five-membered thiadiazole ring is critical to the potency or selectivity observed at PPARs. Consistently, the CF_3 analog **56** and OCF_3 analog **57** in the reverse-thiadiazole series displayed 5- to 20-fold lower potency at PPARα (EC_{50} = 425 nM for **56** and EC_{50} = 656 nM for **57**, Table 3), and 4- to 19-fold lower potency at PPARδ (EC_{50} = 26 nM for **56** and EC_{50} = 58 nM for **57**) compared to that of **22** and **23** in the thiadiazole series, respectively. In addition, both 4-Cl analog **62** and 3-Cl,4-Cl analog **66** in the reverse-thiadiazole series behaved as PPARδ selective agonists only (EC_{50} = 237 nM for **62** and EC_{50} = 229 nM for **66**), with no activity observed at PPARα or PPARγ. Based on these four examples, it is suggested that the orientation of [1,2,4]thiadiazole analogs may have more favorable interactions with the ligand-binding pockets of PPARα and PPARδ than that of the reverse-[1,2,4]thiadiazole analogs shown in Table 3. We also briefly examined the impact of chain length of the molecules on PPAR activities. Insertion of a additional methylene unit into **22** gave **70**. Compound **70** showed higher potency at PPARα (EC_{50} = 37 nM, 65%, Table 4), higher potency at PPARδ (EC_{50} = 2 nM, 66%), and slightly higher activity at PPARγ (EC_{50} = 465 nM, 35%) compared to **22** (Table 2). The two-carbon analog of **40** (**75**) was also prepared. Interestingly, **75** displayed similar potency at PPARα (EC_{50} = 123 nM, 71%), similar potency at PPARδ (EC_{50} = 9 nM, 71%), and higher potency at

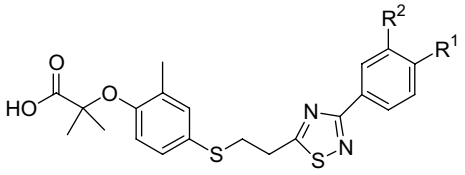
Table 3. Activities in human PPAR co-transfection assays



Compound	R^1	R^2	EC_{50}[a] (nM)		
			PPARα (% resp.)	PPARδ (% resp.)	PPARγ (% resp.)
56	CF_3	H	425 (38)	26 (67)	>3000
57	OCF_3	H	656 (47)	58 (55)	>3000
62	Cl	H	>3000	237 (62)	>3000
66	Cl	Cl	>3000	229 (58)	>3000

[a] EC_{50} is the concentration of test compounds needed to induce 50% of the maximum luciferase activity. The EC_{50} value is the average of more than two separate tests.

Table 4. Activities in human PPAR co-transfection assays



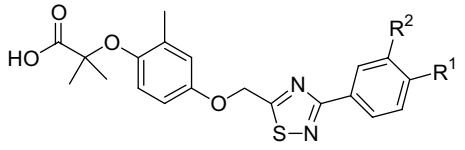
Compound	R^1	R^2	EC_{50}[a] (nM)		
			PPARα (% resp.)	PPARδ (% resp.)	PPARγ (% resp.)
70	CF_3	H	37 (65)	2 (66)	465 (35)
75	Cl	Cl	123 (71)	9 (71)	815 (21)

[a] EC_{50} is the concentration of test compounds needed to induce 50% of the maximum luciferase activity. The EC_{50} value is the average of more than two separate tests.

PPARγ (EC_{50} = 815 nM, 21%) compared to **40** (Table 2). These two examples demonstrate that elongation of chain length might be the direction to pursue if PPAR pan agonists are desired.

Since oxygen may behave as a bioisostere of sulfur in some cases, we were interested in synthesizing oxygen-link analogs. The oxygen-link 4-CF_3 analog **76** displayed comparable potency with higher activity at PPARα (EC_{50} = 94 nM, 71%, Table 5), higher potency at PPARδ (EC_{50} = 3 nM, 79%), but abolished activity at PPARγ (EC_{50} > 3000 nM) compared to that of sulfur-link CF_3 analog **22**. The oxygen-link OCF_3 analog **77** showed ~3-fold lower potency but higher activity at PPARα (EC_{50} = 94 nM, 80%), 4-fold lower potency but higher activity at PPARδ (EC_{50} = 12 nM, 83%) compared to sulfur-link **23**. Based on these two examples, it seems the oxygen-link analogs exhibited slightly lower potency but stronger activity at PPARα. Since we learned that the 3,4-disubstituted analogs tend to exhibit higher potency at PPARα than the corresponding 4-substituted analogs in the sulfur-link series (Table 2), we prepared several 3,4-disubstituted analogs in the oxygen-link series with the hope to increase the potency at PPARα as well. However, while 3-F,4-CF_3 analog **79** displayed modest improvement of potency at PPARα (EC_{50} = 86 nM, 65%) over 4-CF_3 analog **76** (EC_{50} = 94 nM, 71%), 3-Cl,4-OCF_3 analog **80** only improved the activity at PPARα (EC_{50} = 97 nM, 101%) compared to the corresponding 4-OCF_3 analog **77** (EC_{50} = 94 nM, 80%). On the other hand, the oxygen-link 3,4-Cl_2 analog **81**, 3,4-$(CH_3)_2$ analog **82**, and 3-Cl,4-CH_3 analog **83** all displayed 4- to 6-fold lower potency but higher activity at PPARα (EC_{50} = 504 nM, 83% for **81**, 643 nM, 58% for **82**, and 297 nM, 75% for **83**) and 6- to 14-fold lower potency at PPARδ (EC_{50} = 114 nM, 75% for **81**, 534 nM, 60% for **82**, and 148 nM, 85% for **83**) than the corresponding sulfur-link analogs. Overall, replacement of the sulfur atom with the oxygen atom in the linker, in general, resulted in the reduced potency at both PPARα and PPARδ, but increased activity at PPARα. This replacement also displayed either decreased potency or no impact at PPARγ. In order to confirm the critical role for potency of the methyl group on the left-hand-side phenyl ring, we prepared the des-methyl analog of **22**. Indeed, **86** showed 2-fold lower potency and weaker activity at PPARα (EC_{50} = 155 nM, 31%, Table 6), ~3-fold lower potency at PPARδ (EC_{50} = 20 nM, 70%), and abolished activity at PPARγ (EC_{50} > 3000 nM) compared to **22**. Finally, we wished to examine the steric tolerance of substituents at the α-position of the critical carboxyl group beyond the geminal-dimethyl group. Therefore, a cyclopentyl group was installed, and compound **87** displayed 22-fold reduced potency at PPARα (EC_{50} = 736 nM, 31%), over 333-fold decreased potency at PPARδ (EC_{50} > 1000 nM) compared to **23** (Table 2). This detrimental effect was further confirmed in 3,4-dichloro analog **89** where the activities at all three PPAR subtypes were significantly diminished (EC_{50} > 3000 nM for PPARα, >1000 nM for PPARδ, and >3000 nM for PPARγ). In addition, there was no cross-activity with representative compounds, such as **23** and **40** against several nuclear hormone receptors such as farnesoid X receptor (FXR), liver X receptor (LXR), retinoic acid X receptor (RXR), glucocorticoid receptor (GR), and retinoic acid receptor (RAR). In summary, when compared to PPARδ selective agonist **5**, these [1,2,4]thiadiazole derivatives appear as a new series of potent and selective PPARα/δ dual agonists.

Table 5. Activities in human PPAR co-transfection assays

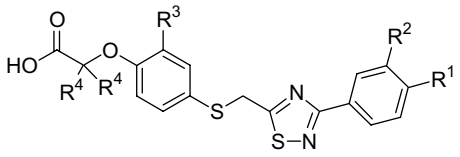


Compound	R^1	R^2	EC_{50}[a] (nM)		
			PPARα (% resp.)	PPARδ (% resp.)	PPARγ (% resp.)
76	CF_3	H	94 (71)	3 (79)	>3000
77	OCF_3	H	94 (80)	12 (83)	>3000
79	CF_3	F	86 (65)	3 (76)	>3000
80	OCF_3	Cl	97 (101)	13 (79)	>3000
81	Cl	Cl	504 (83)	114 (75)	>3000
82	CH_3	CH_3	643 (58)	534 (60)	>3000
83	CH_3	Cl	297 (75)	148 (85)	>3000

[a] EC_{50} is the concentration of test compounds needed to induce 50% of the maximum luciferase activity. The EC_{50} value is the average of more than two separate tests.

To determine the activity of these compounds on the endogenous human receptor, we employed real time PCR to measure the expression levels of a panel of genes involved in lipid metabolism and energy expenditure. The cell types utilized in these experiments were primary human skeletal muscle cells. To date, **40** induced several target genes in a dose-dependent manner (Fig. 2). For example, a robust induction of ATP-binding cassette transporter A1 (ABC-A1)[23] by **40** was detected in skeletal muscles (1.5-fold increase at 1 nM to 4.8-fold increase at 1000 nM). Since ABC-A1 is involved in lipid and protein metabolism, the induction of this gene by **40** suggests PPARα/δ dual agonists may play an important role in this function. Meanwhile, a significant induction of carnitine palmitoyltransferase type 1 (CPT-1)[24] by **40** (3-fold at 1 nM to 7.8-fold at 1000 nM) suggests **40** may be involved in fatty acid oxi-

Table 6. Activities in human PPAR co-transfection assays



Compound	R^1	R^2	R^3	R^4	EC_{50}[a] (nM)		
					PPARα (% resp.)	PPARδ (% resp.)	PPARγ (% resp.)
86	CF_3	H	H	CH_3	155 (31)	20 (70)	>3000
87	OCF_3	H	CH_3		736 (31)	>1000	>3000
89	Cl	Cl	CH_3		>3000	>1000	>3000

[a] EC_{50} is the concentration of test compounds needed to induce 50% of the maximum luciferase activity. The EC_{50} value is the average of more than two separate tests.

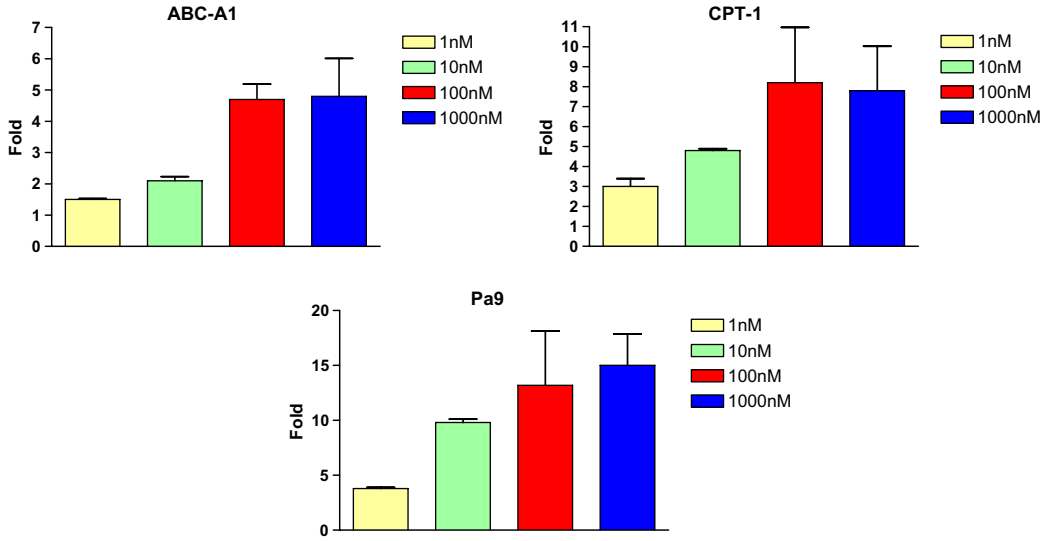


Figure 2. Induction of selected target genes by **40** in primary, human skeletal muscle cells. Genes include: ABCA1, ATP binding cassette transporter A1; CPT-1, Carnitine palmitoyl transferase type I; and Pa9, PPARα in-house target gene. Fold increase over vehicle was expressed as mean ± SD (standard error mean) of triplicate samples.

dation. In addition, a strong induction of a new PPARα target gene, Pa9,[25] was also observed (3.8-fold at 1 nM to 15-fold at 1000 nM). While the monosubstituted 4-OCF_3-analog **23** was found to have P450 induction issue at 10 μM concentration, we were pleased to find that the disubstituted 3,4-dichloro-analog **40** displayed only modest P450 induction at the same concentration. However, the reason for the different degree of P450 induction caused by the subtle change in chemical structure is unclear at this point. Meanwhile, in human liver microsome cytochrome P450 inhibition assay, **40** displayed $IC_{50} > 30$ μM for all subtypes tested. Therefore, it should have low risk for potential drug–drug interactions.

3.2. In vivo studies

The ability of compound **40** to affect lipid/cholesterol homeostasis in vivo was first examined in a hypercholesterolemic rat model. While the high-cholesterol diet fed hypercholesterolemic rat displayed about 2.8-fold lower HDL-C level (17.7 mg/dL, Fig. 3) than the standard chow diet fed rat (49 mg/dL), compound **40** increased the serum HDL-C level (from 17.7 to 45 mg/dL) in a dose-related manner after these rats were dosed orally for 8 days. In addition, while this hypercholesterolemic rat displayed about 8.7-fold elevated LDL-C level (96 mg/dL, Fig. 3) than that of the standard chow diet fed rat (11 mg/dL), **40** attenuated the high LDL-C level (from 96 to 70 mg/dL) in a dose-related manner. This LDL-C lowering effect of **40** seems more related to PPARα agonist rather than PPARδ agonist property. When dosed from 0.1 to 1 mg/kg/d, compound **40** dose-dependently decreased the total cholesterol from 423 to 263 mg/dL. Similar to PPARα agonists, there was also a dose-related decrease in triglycerides observed when dosed with **40**. A pharmacokinetic (PK) arm of this study is shown in Table 7. The C_{max} and

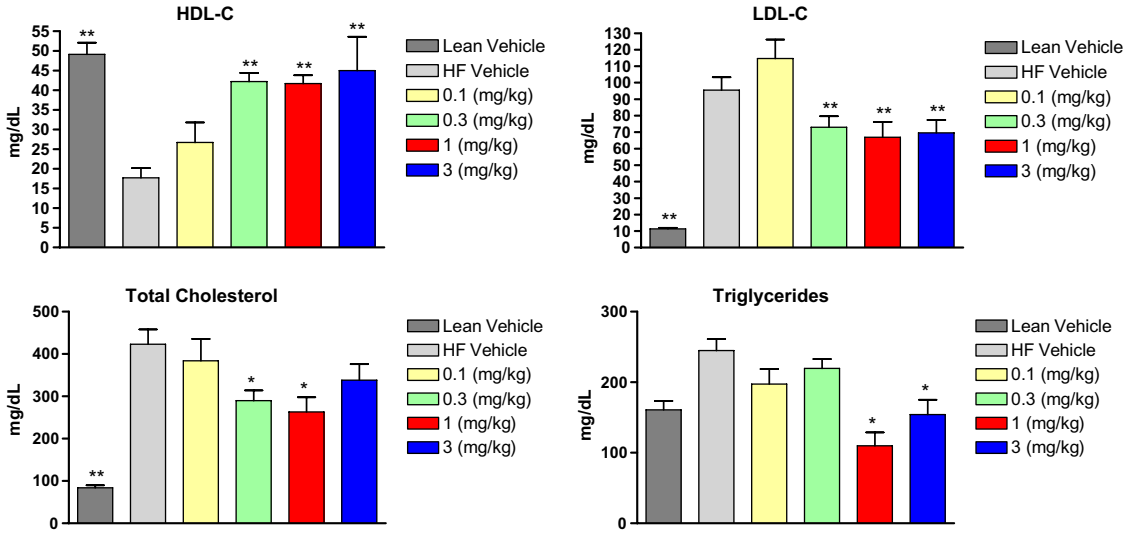


Figure 3. Effects of **40** on HDL-C, LDL-C, total cholesterol, and triglyceride levels in hypercholesterolemic rats (8 days oral dosing). Bars represent means ± SD (n = 8 rats per group; $^{*}p < 0.05$, $^{**}p < 0.01$ relative to vehicle HF).

AUC increased proportionally with dose. High plasma drug exposure (e.g., AUC = 13,900 ng h/mL at 1 mg/kg) and long plasma duration ($t_{1/2}$ = 5.7 h at 1 mg/kg) was observed, no unexpected accumulation occurred after multiple dosing.

Table 7. PK parameters in Sprague–Dawley rats following either multiple (8 days of treatment) or a single dose of **40**

Dose (mg/kg)	Interval	C_{max} (ng/mL)	AUC (ng h/mL)	$t_{1/2}$
0.1	Multiple	82 ± 18	738 ± 1	5.5
0.3	Multiple	231 ± 16	2401 ± 284	5.1
1	Multiple	1361 ± 542	13,900 ± 4051	5.7
3	Multiple	2004 ± 425	27,124 ± 4284	7.1
3	Single	2521 ± 1204	24,241 ± 11,415	7.2

Data are expressed as means ± SD, N = 2–3 per group. For the single dose group, the animals were treated with vehicle for 7 days and administered a single dose of **40** on day 8.

The capability of **40** to affect lipid homeostasis was next examined in a human apoA1 transgenic mouse model. Since apoA1 is the primary protein component of HDL-C, a human apoA1 transgenic mouse model has proven useful in the testing of PPARα and PPARδ hypolipidemic compounds.[26] Male hApoA1 transgenic mice were dosed orally with **40** for 7 days (Fig. 4). Compound **40** dose-dependently (from 0.3 to 3 mg/kg) increased serum HDL-C levels, which paralleled the increases in serum hApoA1 levels. This result suggests that the compound increased a PPARα target ApoA1 protein, which may contribute to the increase in HDL-C levels.

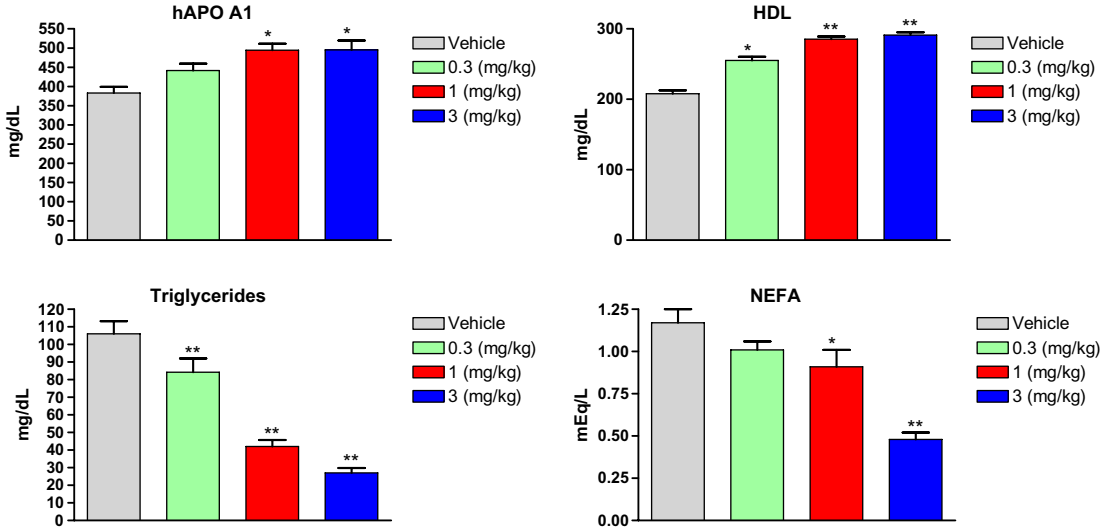


Figure 4. Effects of **40** on hApoA1, high-density lipoprotein, triglyceride, and non-esterified free fatty acid levels in male human Apo A1 transgenic mice (7 days oral dosing). Bars represent means ± SD (n = 8 rats per group; $^{*}p < 0.05$, $^{**}p < 0.01$ relative to vehicle HF).

Compound **40** also significantly reduced triglycerides and decreased free fatty acids.

The ob/ob mice, a genetic leptin deficient model, are obese and insulin resistant with hypertriglyceridemia, hyperglycemia and has been used as a rodent model of obesity-induced insulin resistance. Seven-week-old female ob/ob mice were dosed orally (from 0.1 to 3 mg/kg) with **40** for 11 days (Fig. 5). Compound **40** significantly reduced serum glucose (52% decrease, from 779 to 375 mg/dL), insulin (50% decrease, from 40 to 20 ng/mL), and triglycerides (83% decrease, from 530 to 92 mg/dL). Unlike PPARγ agonists in this model, compound **40** improved insulin sensitivity but reduced the body weight at the same time. However, liver weights increased (65% increase, from 2.6 to 4.3 g) similar to the effect observed with PPARα agonists. A PK arm of this study is shown in Table 8. The C_{max} and AUC increased proportionally up to 1 mg/kg dose. From 1 to 3 mg/kg dose, a minor unexpected accumulation occurred. High plasma drug exposure (e.g., AUC = 47,600 ng h/mL at 1 mg/kg) and long plasma duration ($t_{1/2}$ = 5.2 h at 1 mg/kg) were observed in this ob/ob mice model.

The in vivo antihyperlipidemic activity of **40** was also evaluated in obese, mildly hypertriglyceridemic female Beagle dogs. Animals were dosed with **40** (0.3–10 mg/kg) by oral gavage for 7 days, approximately 3 h before feeding. There was a dose-dependent reduction in serum triglycerides, total cholesterol, and free fatty acids (Fig. 6). From 0.3 to 3 mg/kg doses, there was no change in serum level of liver enzyme at AST (or ALT, data not shown). However, at 10 mg/kg, there was a significant increase (1.7-fold) at AST level. PK arm data are shown in Table 9. The C_{max} and AUC increased proportionally with dose, and no unexpected accumulation occurred after multiple dosing. High plasma drug exposure (e.g., AUC = 24,120 ng h/mL at 3 mg/kg) and long plasma duration ($t_{1/2}$ = 6 h at 3 mg/kg) were also observed in this female Beagle dog model.

Table 8. PK parameters in ob/ob mice following either multiple (11 days of treatment) or a single dose of **40**

Dose (mg/kg)	Interval	C_{max} (ng/mL)	AUC (ng h/mL)	$t_{1/2}$
0.1	multiple	480 ± 108	2785 ± 553	5.1
0.3	multiple	1836 ± 346	10,595 ± 578	5.0
1	multiple	6830 ± 922	47,600 ± 891	5.2
3	multiple	26,337 ± 6603	248,545 ± 25,829	6.7
3	single	19,343 ± 7914	114,823 ± 2370	5.1

Data are expressed as means ± SD, N = 2–3 per group. For the single dose group, the animals were treated with vehicle for 10 days and administered a single dose of **40** on day 11.

3.3. Molecular modeling

Receptor–ligand docking studies were carried out to help gain insight into the binding mode of compound **21** (Table 1) and ligand subtype selectivity. Figure 7 illustrates a low energy binding mode of **21** in PPARδ as determined by Glide4.5.[27,28] The binding mode is sim-

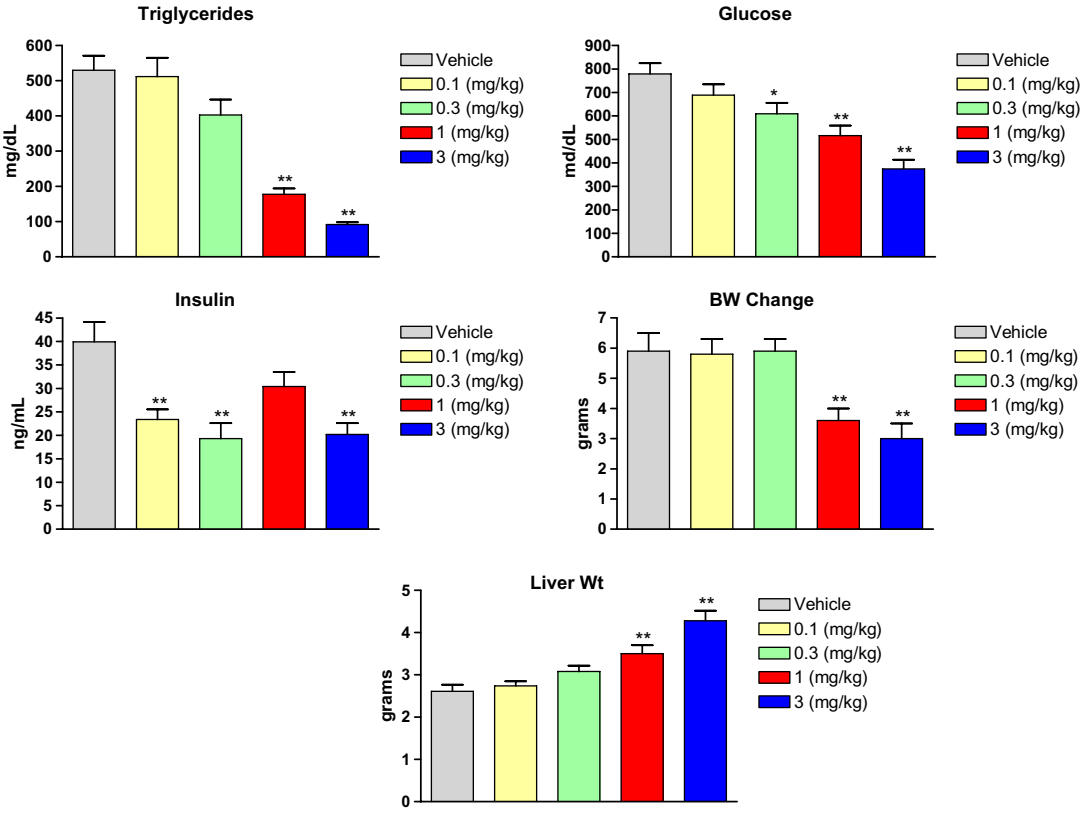


Figure 5. Effects of **40** on triglyceride, glucose, insulin levels, body weight change, and liver weight change in female ob/ob mice (11 days oral dosing). Bars represent means ± SD (n = 8 rats per group; $^{*}p < 0.05$, $^{**}p < 0.01$ relative to vehicle HF).

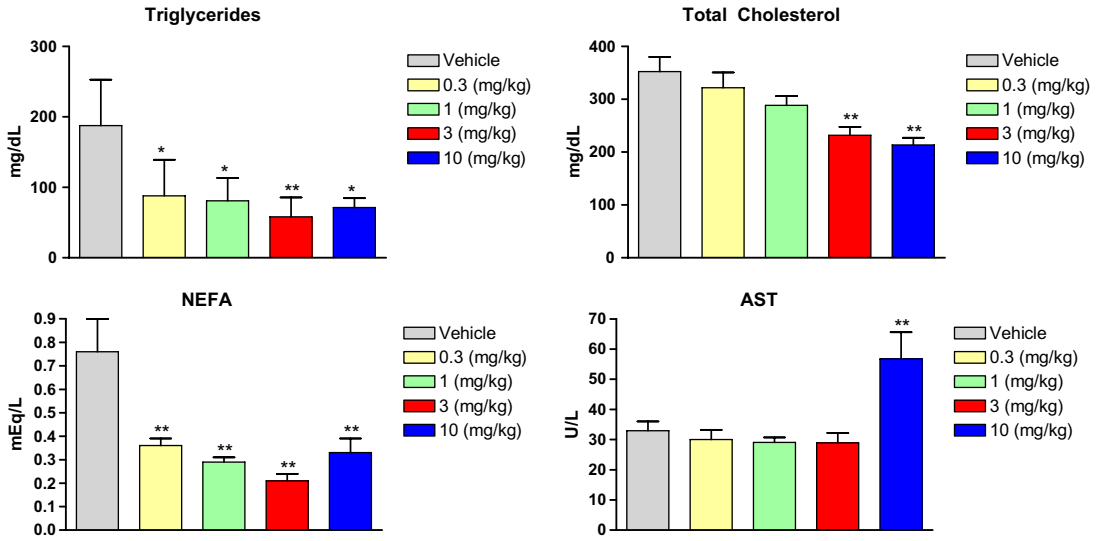


Figure 6. Effects of **40** on triglyceride, total cholesterol, non-esterified fatty acids, and liver enzyme activity levels in female Beagle dogs (7 days oral dosing). Bars represent means ± SD ($n = 8$ rats per group; $^{*}p < 0.05$, $^{**}p < 0.01$ relative to vehicle HF).

Table 9. PK parameters in female Beagle dogs following either multiple (7 days of treatment) or a single dose of **40**

Dose (mg/kg)	Interval	C_{max} (ng/mL)	AUC (ng h/mL)	$t_{1/2}$
0.3	Multiple	398 ± 121	2649 ± 1049	6.5
1	Multiple	1529 ± 84	6505 ± 1984	4.7
3	Multiple	6711 ± 1288	24,120 ± 4631	6.0
10	Multiple	10,068 ± 2215	61,563 ± 24,563	6.2
10	Single	10,138 ± 5637	57,238 ± 35,201	5.8

Data are expressed as means ± SD, $N = 2$–3 per group. For the single dose group, the animals were treated with vehicle for 6 days and administered a single dose of **40** on day 7.

ilar to many PPAR/ligand structures in that an acidic, ligand 'head' group forms hydrogen bonds with residues at the terminus of the so-called Arm I pocket.[29] In our case, the fibrate-like head group of **21** forms a network of hydrogen bonds with H323, Y473, and H449. At the mid-section of Arm I, the ortho substituted methyl group of the phenol occupies a small hydrophobic pocket formed by F282, C285, and I363.[30] In addition, the *para*-trifluoro phenyl extends deep into hydrophobic Arm II. In an attempt to help rationalize PPARα/δ isotype selectivity, the compounds in Table 1 were docked

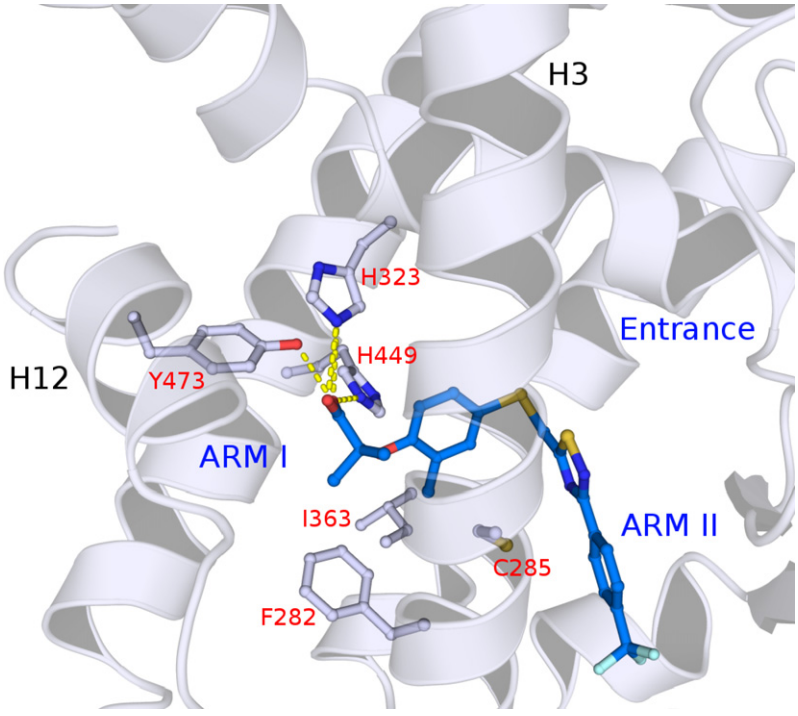


Figure 7. Predicted binding mode of **21** in PPARδ. The carboxylate head group of **21** (blue) forms multiple hydrogen bonds with Y473, H323, and H449 in Arm I. The phenolic ortho-methyl projects into a small hydrophobic pocket outlined by F282, I363, and C285. The para trifluoromethyl phenyl side of the molecule occupies Arm II of the PPARδ ligand binding domain. PPARδ is rendered semi-transparent for ease of view, **21** lies behind H3.

into both PPAR isotypes. Docking experiments, with and without hydrogen bonding constraints, were carried out. Unfortunately, docking energies and binding modes in all experiments revealed little about ligand/PPAR isotype selectivity. These results concur with previous studies whose authors find that PPAR isotype selectivity remains beyond the limits of virtual screening technology and is therefore poorly represented by docking experiments.[31] The barrier to ligand/isotype selectivity in structure-based modeling is primarily due to the following reasons: high conservation of active site residues between the three PPAR isotypes.[32] Approximately 34 residues make up the ligand-binding cavities of the PPARs with at least 80% of these conserved[32]; very large ligand binding cavities in all the PPARs. PPAR receptors have some of the largest (~1400 Å^3) binding pockets identified in nuclear hormone receptor (NHR) ligand binding domains.[29] Finally, the ligand binding domains are very flexible. PPAR receptors have been shown to undergo induced fit upon ligand binding and can bind different conformations of the same ligand.[33]

4. Conclusion

In an attempt to rationalize the unexpected potent and selective PPARα/δ dual agonist activity, **21** was docked into all three PPAR isotypes. Unfortunately, docking energies and binding mode experiments revealed little about SAR selectivity. Nonetheless, compound **40** and its close analogs represent a new series of PPARα/δ dual agonist. The high potency, high selectivity, significant gene induction, excellent PK profile, low P450 inhibition or induction, and good in vivo efficacy in four animal models support **40** being selected as a pre-clinical study candidate, and may render **40** as valuable pharmacological tool in elucidating the complex roles of PPARα/δ dual agonists, and the potential usage for the treatment of metabolic syndrome.

5. Experimental

5.1. Chemical synthesis

5.1.1. General remarks. ^{1}H NMR spectra were measured on a Bruker AC-300 (300 MHz) spectrometer using tetramethylsilane as an internal standard. Elemental analyses were obtained by Quantitative Technologies Inc. (Whitehouse, New Jersey), and the results were within 0.4% of the calculated values unless otherwise mentioned. Melting points were determined in open capillary tubes with a Thomas–Hoover apparatus and were uncorrected. Electrospray mass spectra (MS-ES) were recorded on a Hewlett Packard 59987A spectrometer. High resolution mass spectra (HRMS) were obtained on a Micromass Autospec. E. spectrometer. The term 'DMAP' refers to dimethylamino-pyridine, 'TFA' refers to trifluoroacetic acid, 'NMP' refers to 1-methyl-2-pyrrolidinone, 'DPPA' refers to diphenylphosphoryl azide. The syntheses of compounds **21**, **22**, **23**, **24**, **56**, **57**, **76**, **77**, and **87** were described in preliminary report.[17]

5.1.2. 4-(4-Trifluoromethyl-phenyl)-but-3-yn-2-one (2). To a solution of **1** (1.25 g, 7.36 mmol) at room temperature was added 1.0 M EtMgBr (8.8 mL, 8.8 mmol) in *tert*-butyl methyl ether. After heating at 35 °C for 2 h, the solution was cooled to room temperature and then transferred via a cannula to another flask containing acetic anhydride (1.4 mL, 14.9 mmol) and THF (5 mL) at 4 °C. The solution was stirred at 4 °C and warmed to room temperature overnight. Saturated NH_4Cl was added, and the organic phase was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried, concentrated, and the product was purified by column chromatography (EtOAc/hexane) to give 1.46 g (94%) of **2** as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, J = 8.8 Hz, 2H), 7.65 (d, J = 8.9 Hz, 2H), 2.48 (s, 3H).

5.1.3. 3-Methyl-5-(4-trifluoromethyl-phenyl)-isothiazole (3). A mixture of **2** (435 mg, 2.05 mmol) and hydroxylamine-*O*-sulfonic acid (245 mg, 2.17 mmol) in water (0.8 mL) was stirred at room temperature for 30 min. Solid $NaHCO_3$ (183 mg, 2.18 mmol) and water (0.5 mL) were added and the mixture was stirred for 15 min. NaSH (1.0 M) (2.3 mL, 2.3 mmol) in water was added and the mixture was stirred overnight and extracted with CH_2Cl_2. The extracts were dried, concentrated, and the product was purified by column chromatography to give 347 mg (70%) of **3** as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (s, 4H), 7.24 (s, 1H), 2.55 (s, 3H); MS (ES) m/z: 244 ($M+H^+$).

5.1.4. 3-Bromomethyl-5-(4-trifluoromethyl-phenyl)-isothiazole (4). A mixture of **3** (110 mg, 0.453 mmol), *N*-bromosuccinimide (88 mg, 0.49 mmol), and benzoyl peroxide (11 mg, 0.045 mmol) in CCl_4 (4 mL) was refluxed for 18 h, and then diluted with CH_2Cl_2 and water. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried, concentrated, and column chromatographed (CH_2Cl_2/hexane) to provide 61 mg (42%) of **4** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (s, 4H), 7.53 (s, 1H), 4.58 (s, 2H).

5.1.5. {2-Methyl-4-[5-(4-trifluoromethyl-phenyl)-isothiazol-3-ylmethylsulfanyl]-phenoxy}-acetic acid (6). A mixture of **4** (60 mg, 0.18 mmol) and (4-mercapto-2-methyl-phenoxy)acetic acid ethyl ester **5** (63 mg, 0.28 mmol) in CH_3CN (3 mL) was degassed under N_2 for about 15 min. After addition of Cs_2CO_3 (91 mg, 0.28 mmol), the mixture was stirred overnight under N_2, concentrated, and purified by column chromatography (EtOAc/hexane) to give 67 mg (77%) of 6-ethyl ester as white crystals: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.6 Hz, 2H), 7.31 (s, 1H), 7.19 (d, J = 1.8 Hz, 1H), 7.14 (dd, J = 8.4, 2.2 Hz, 1H), 6.60 (d, J = 8.4 Hz, 1H), 4.61 (s, 2H), 4.25 (q, J = 7.1 Hz, 2H), 4.16 (s, 2H), 2.23 (s, 3H), 1.28 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 468 ($M+H^+$). Anal. calcd for $C_{22}H_{20}F_3NO_3S_2$: C, 56.52; H, 4.31; N, 3.00. Found: C, 56.54; H, 3.86; N, 3.00. A mixture of 6-ethyl ester (54 mg, 0.12 mmol) and 2 M NaOH (0.2 mL, 0.4 mmol) in MeOH (1.5 mL) was stirred under N_2 for 2 h and

concentrated. CH_2Cl_2 and water were added, and the mixture was acidified with concentrated HCl. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried, concentrated, and column chromatographed (0.3% AcOH in EtOAc) to give 49 mg (97%) of **6** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, J = 8.7 Hz, 2H), 7.65 (d, J = 8.6 Hz, 2H), 7.35 (s, 1H), 7.18 (s, 1H), 7.16 (m, 1H), 6.64 (d, J = 8.2 Hz, 1H), 4.66 (s, 2H), 4.17 (s, 2H), 2.22 (s, 3H); MS (ES) *m/z*: 440 ($M+H^+$). Anal. calcd for $C_{20}H_{16}F_3NO_3S_2 \cdot 0.6H_2O$: C, 53.35; H, 3.85; N, 3.11. Found: C, 53.20; H, 3.51; N, 2.91.

5.1.6. 3-(4-Trifluoromethyl-phenyl)-isothiazole-5-carboxylic acid ethyl ester (8). The reaction mixture of **7** (608 mg, 2.46 mmol) and ethyl propiolate (726 mg, 7.41 mmol) in chlorobenzene (10 mL) was heated at 135 °C for 20 h. TLC showed some of the starting material **7** still remained. More ethyl propiolate (726 mg, 7.41 mmol) and 1,2-dichlorobenzene (10 mL) were added and the solution was heated at 160 °C for 7 h. After cooling to room temperature, the reaction mixture was concentrated and the product was purified by column chromatography to give 264 mg (36%) of **11** as an off-white solid and 229 mg (31%) of **8** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.09 (d, J = 8.1 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H), 4.44 (q, J = 7.1 Hz, 2H), 1.43 (t, J = 7.1 Hz, 3H); MS (ES) *m/z*: 302 ($M+H^+$).

5.1.7. [3-(4-Trifluoromethyl-phenyl)-isothiazol-5-yl]-methanol (9). To the solution of **8** (104 mg, 0.345 mmol) in THF (2 mL) at −78 °C was added 1.0 M $LiAlH_4$ (0.21 mL, 0.21 mmol) in THF. After stirring at −78 °C for 30 min, water was slowly added and the mixture was allowed to warm up to room temperature. The precipitated solid was filtered and rinsed with CH_2Cl_2. The filtrate was washed with saturated NH_4Cl, and the aqueous solution was back extracted with CH_2Cl_2. The combined organic phases were dried and concentrated to give 98 mg of **9** as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, J = 8.3 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.51 (s, 1H), 5.06 (s, 2H); MS (ES) *m/z*: 260 ($M+H^+$).

5.1.8. {2-Methyl-4-[3-(4-trifluoromethyl-phenyl)-isothiazol-5-ylmethylsulfanyl]-phenoxy}-acetic acid (10). To a solution of **9** (98 mg, 0.38 mmol) in CH_2Cl_2 (3 mL) were added methanesulfonyl chloride (59 mg, 0.52 mmol) and triethylamine (0.096 mL, 0.69 mmol). The solution was stirred at room temperature overnight and diluted with CH_2Cl_2 and water. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic phases were dried and concentrated to provide 95 mg of the crude mesylate as a yellow solid. A mixture of the crude mesylate (95 mg) and (4-mercapto-2-methyl-phenoxy)acetic acid ethyl ester **5** (115 mg, 0.51 mmol) in CH_3CN (2 mL) was degassed under N_2 for about 15 min. After addition of Cs_2CO_3 (220 mg, 0.67 mmol), the mixture was stirred for 4 h under N_2, concentrated, and the product was purified by column chromatography (EtOAc/hexane) to give 66 mg (41%, 3 steps) of **10-ethyl ester** as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, J = 8.2 Hz, 2H), 7.68 (d, J = 8.3 Hz, 2H), 7.30 (s, 1H), 7.25 (d, J = 2.1 Hz, 1H), 7.17 (dd, J = 8.4, 2.2 Hz, 1H), 6.61 (d, J = 8.4 Hz, 1H), 4.62 (s, 2H), 4.25 (s, 2H), 4.24 (q, J = 7.1 Hz, 2H), 2.24 (s, 3H), 1.27 (t, J = 7.1 Hz, 3H); MS (ES) *m/z*: 490 ($M+Na^+$). A mixture of 10-ethyl ester (56 mg, 0.12 mmol) and 2 M NaOH (0.15 mL, 0.30 mmol) in MeOH (2.5 mL) was stirred under N_2 for 2 h and concentrated. CH_2Cl_2 and water were added, and the mixture was acidified with concentrated HCl. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried, concentrated, and column chromatographed (0.3% AcOH in EtOAc) to give 50 mg (95%) of **10** as a yellow oil which solidified upon standing over time: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 8.1 Hz, 2H), 7.67 (d, J = 8.2 Hz, 2H), 7.32 (s, 1H), 7.24 (d, J = 1.7 Hz, 1H), 7.19 (dd, J = 8.4, 2.2 Hz, 1H), 6.63 (d, J = 8.4 Hz, 1H), 4.67 (s, 2H), 4.26 (s, 2H), 2.23 (s, 3H); MS (ES) *m/z*: 440 ($M+H^+$) Anal. calcd for $C_{20}H_{16}F_3NO_3S_2$: C, 54.66; H, 3.67; N, 3.19. Found: C, 54.30; H, 3.52; N, 3.06.

5.1.9. 3-(4-Trifluoromethyl-phenyl)-isothiazole-4-carboxylic acid ethyl ester (11). From the same reaction that provided **8** as shown above, 264 mg (36%) of **11** was also isolated as an off-white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.39 (s, 1H), 7.76 (d, J = 8.3 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 4.28 (q, J = 7.1 Hz, 2H), 1.27 (t, J = 7.1 Hz, 3H); MS (ES) *m/z*: 302 ($M+H^+$).

5.1.10. [3-(4-Trifluoromethyl-phenyl)-isothiazol-4-yl]-methanol (12). To the solution of **11** (96 mg, 0.32 mmol) in EtOH (4 mL) at room temperature was added $NaBH_4$ (30 mg, 0.79 mmol). After overnight, more $NaBH_4$ (154 mg, 4.05 mmol) and EtOH (4 mL) were added and stirring was continued for 24 h. The solvent was evaporated, and the residue was partitioned between CH_2Cl_2 and water. The organic phase was dried and concentrated to provide 77 mg (93%) of **12** as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.76 (s, 1H), 7.90 (d, J = 8.1 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H), 4.81 (s, 2H); MS (ES) *m/z*: 260 ($M+H^+$).

5.1.11. {2-Methyl-4-[3-(4-trifluoromethyl-phenyl)-isothiazol-4-ylmethylsulfanyl]-phenoxy}-acetic acid (13). The mixture of **12** (77 mg, 0.30 mmol), methanesulfonyl chloride (53 mg, 0.47 mmol), and triethylamine (0.083 mL, 0.60 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature overnight. The solution was washed with water and the aqueous phase was back extracted with CH_2Cl_2. The combined organic layers were dried and concentrated to provide 107 mg of the crude mesylate as an off-white solid. A mixture of the crude mesylate (107 mg) and (4-mercapto-2-methyl-phenoxy)acetic acid ethyl ester **5** (90 mg, 0.40 mmol) in CH_3CN (2 mL) was degassed under N_2 for about 15 min. After addition of Cs_2CO_3 (167 mg, 0.512 mmol), the mixture was stirred for 4 h under N_2, concentrated, and purified by column chromatography (EtOAc/hexane) to give 114 mg (77%, 2 steps) of 13-ethyl ester as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.80 (d, J = 8.2 Hz, 2H), 7.72 (d, J = 8.3 Hz, 2H),

7.11 (d, J = 1.7 Hz, 1H), 7.05 (dd, J = 8.4, 2.2 Hz, 1H), 6.58 (d, J = 8.4 Hz, 1H), 4.63 (s, 2H), 4.27 (q, J = 7.1 Hz, 2H), 4.04 (s, 2H), 2.23 (s, 3H), 1.30 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 490 ($M+Na^+$). A mixture of 13-ethyl ester (103 mg, 0.221 mmol) and 2 M NaOH (0.30 mL, 0.60 mmol) in MeOH (2.5 mL) was stirred under N_2 for 2 h and concentrated. CH_2Cl_2 and water were added, and the mixture was acidified with concentrated HCl. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried, concentrated, and column chromatographed (0.3% AcOH in EtOAc) to give 90 mg (93%) of **13** as a white solid: ^1H NMR (300 MHz, MeOH-d_4) δ 8.56 (s, 1H), 7.75 (s, 4H), 7.01 (s, 1H), 6.99 (m, 1H), 6.66 (d, J = 8.2 Hz, 1H), 4.67 (s, 2H), 4.10 (s, 2H), 2.14 (s, 3H); MS (ES) m/z: 440 ($M+H^+$). Anal. calcd for $C_{20}H_{16}F_3NO_3S_2$: C, 54.66; H, 3.67; N, 3.19. Found: C, 54.54; H, 3.53; N, 3.01.

5.1.12. 5-Chloromethyl-3-(4-trifluoromethyl-phenyl)-isoxazole (15). To a mixture of **14** (500 mg, 2.24 mmol) and propargyl chloride (235 mg, 3.15 mmol) in CH_2Cl_2 (12 mL) at 4 °C was added Et_3N (0.38 mL, 2.73 mmol). After removing of the cooling bath, the reaction mixture was stirred at room temperature for 5 h and then washed with water. The aqueous phase was back extracted with CH_2Cl_2, and the combined organic layers were dried, concentrated and column chromatographed to give 373 mg (64%) of **15** as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, J = 8.2 Hz, 2H), 7.73 (d, J = 8.3 Hz, 2H), 6.68 (s, 1H), 4.68 (s, 2H); MS (ES) m/z: 262 ($M+H^+$).

5.1.13. {2-Methyl-4-[3-(4-trifluoromethyl-phenyl)-isoxazol-5-ylmethylsulfanyl]-phenoxy}-acetic acid (16). A mixture of **15** (262 mg, 1.00 mmol) and **5** (340 mg, 1.50 mmol) in CH_3CN (8 mL) was degassed under N_2 for about 15 min. After addition of Cs_2CO_3 (490 mg, 1.50 mmol), the reaction mixture was stirred for 4 h under N_2, then concentrated, and purified by column chromatography (EtOAc/hexane) to give 410 mg (91%) of 16-ethyl ester as a clear oil which solidified upon standing: ^1H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, J = 8.1 Hz, 2H), 7.70 (d, J = 8.3 Hz, 2H), 7.24 (d, J = 2.7 Hz, 1H), 7.18 (dd, J = 8.4, 2.4 Hz, 1H), 6.61 (d, J = 8.4 Hz, 1H), 6.30 (s, 1H), 4.62 (s, 2H), 4.24 (q, J = 7.1 Hz, 2H), 4.08 (s, 2H), 2.25 (s, 3H), 1.28 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 474 ($M+Na^+$). Anal. calcd for $C_{22}H_{20}F_3NO_4S$: C, 58.53; H, 4.47; N, 3.10. Found: C, 58.51; H, 4.08; N, 2.94. A mixture of 16-ethyl ester (348 mg, 0.772 mmol) and 2 M NaOH (0.4 mL, 0.8 mmol) in water (0.4 mL) and MeOH (11 mL) was stirred under N_2 for 3 h and concentrated. CH_2Cl_2 and water were added, and the mixture was acidified with concentrated HCl. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried and concentrated to give 334 mg (100%) of **16** as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, J = 8.1 Hz, 2H), 7.70 (d, J = 8.0 Hz, 2H), 7.25 (s, 1H), 7.20 (dd, J = 8.3, 1.9 Hz, 1H), 6.65 (d, J = 8.3 Hz, 1H), 6.34 (s, 1H), 4.68 (s, 2H), 4.10 (s, 2H), 2.24 (s, 3H); MS (ES) m/z: 446 ($M+Na^+$). HRMS calcd for $C_{20}H_{16}F_3NO_4S$: 423.0752. Found: 423.0763.

5.1.14. 5-(4-Trifluoromethyl-phenyl)-isoxazole-3-carboxylic acid ethyl ester (18). To the solution of **17** (23.0 g, 79.9 mmol) in EtOH (400 mL) was added hydroxylamine hydrochloride (16.65 g, 240 mmol). After stirring at room temperature for 2 h, the reaction mixture was refluxed for 5 h. The cooled reaction mixture was concentrated under reduced pressure, and the residue was partitioned between CH_2Cl_2 and water. The organic layer was dried, concentrated, and column chromatographed to provide 20.8 g (91%) of **18** as a yellow solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.94 (d, J = 8.2 Hz, 2H), 7.77 (d, J = 8.3 Hz, 2H), 7.04 (s, 1H), 4.49 (q, J = 7.1 Hz, 2H), 1.45 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 286 ($M+H^+$). Anal. calcd for $C_{13}H_{10}F_3NO_3$: C, 54.74; H, 3.53; N, 4.91. Found: C, 54.89; H, 3.20; N, 4.55.

5.1.15. [5-(4-Trifluoromethyl-phenyl)-isoxazol-3-yl]-methanol (19). To a solution of **18** (19.4 g, 68.1 mmol) in THF (420 mL) at −78 °C was added 1.0 M $LiAlH_4$ (41.0 mL, 41.0 mmol) in THF. After stirring at −78 °C for 1 h, water (4 mL) was slowly added and the mixture was allowed to warm to room temperature. After the addition of brine, the precipitated solid was filtered, rinsed with CH_2Cl_2, refluxed in THF (200 mL) two times, and filtered again. The combined filtrates were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried, concentrated, and column chromatographed (EtOAc/hexane) to give 7.45 g (45%) of **19** as a yellow solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, J = 8.2 Hz, 2H), 7.72 (d, J = 8.5 Hz, 2H), 6.69 (s, 1H), 4.83 (s, 2H); MS (ES) m/z: 266 ($M+Na^+$). Anal. calcd for $C_{11}H_8F_3NO_2$: C, 54.33; H, 3.32; N, 5.76. Found: C, 54.38; H, 3.08; N, 5.77.

5.1.16. {2-Methyl-4-[5-(4-trifluoromethyl-phenyl)-isoxazol-3-ylmethylsulfanyl]-phenoxy}-acetic acid (20). To a solution of **19** (7.07 g, 29.1 mmol) in CH_2Cl_2 (320 mL) at 4 °C were added methanesulfonyl chloride (4.44 g, 38.8 mmol) and triethylamine (6.0 mL, 43.1 mmol). The cooling bath was removed and the solution was stirred at room temperature for 3 h. After removal of about two-thirds of CH_2Cl_2 under reduced pressure, Et_2O was added. The precipitated solid was filtered and rinsed with Et_2O. The filtrate was washed with water, and the aqueous solution was back extracted with CH_2Cl_2. The combined organic layers were dried and concentrated to provide 9.19 g of the crude mesylate as a brown solid. A mixture of the crude mesylate (9.19 g) and **5** (8.50 g, 37.6 mmol) in CH_3CN (200 mL) was degassed under N_2 for about 15 min. After addition of Cs_2CO_3 (14.0 g, 42.9 mmol), the mixture was stirred for 2.5 days under N_2 and concentrated. The residue was diluted with CH_2Cl_2 and filtered. The filtrate was concentrated and purified by column chromatography (EtOAc/hexane) to give 10.41 g (79%, 2 steps) of 20-ethyl ester as a yellow solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, J = 8.2 Hz, 2H), 7.71 (d, J = 8.3 Hz, 2H), 7.23 (d, J = 1.8 Hz, 1H), 7.17 (dd, J = 8.4, 2.2 Hz, 1H), 6.61 (d, J = 8.4 Hz, 1H), 6.53 (s, 1H), 4.61 (s, 2H), 4.25 (q, J = 7.1 Hz, 2H), 4.05 (s, 2H), 2.24 (s, 3H), 1.28 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 474 ($M+Na^+$). Anal. calcd for $C_{22}H_{20}F_3NO_4S$: C, 58.53; H, 4.47; N, 3.10.

Found: C, 58.46; H, 4.30; N, 3.01. A mixture of 20-ethyl ester (5.66 g, 12.5 mmol) and 2 M NaOH (6.6 mL, 13.2 mmol) in water (10 mL) and MeOH (160 mL) was stirred under N_2 for 2 h and concentrated. CH_2Cl_2 and water were added, and the mixture was acidified with concentrated HCl (1.2 mL). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried, concentrated, and column chromatographed (0.3% AcOH in EtOAc) to give 5.24 g (99%) of **20** as an off-white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, J = 8.2 Hz, 2H), 7.71 (d, J = 8.3 Hz, 2H), 7.22 (d, J = 1.6 Hz, 1H), 7.17 (dd, J = 8.4, 2.1 Hz, 1H), 6.63 (d, J = 8.4 Hz, 1H), 6.56 (s, 1H), 4.65 (s, 2H), 4.05 (s, 2H), 2.22 (s, 3H); MS (ES) m/z: 446 ($M+Na^+$). Anal. calcd for $C_{20}H_{16}F_3NO_4S$: C, 56.73; H, 3.81; N, 3.31. Found: C, 56.76; H, 3.46; N, 3.20.

5.1.17. General procedure for the synthesis of 26, 32, 37, 42, 47, and 52

5.1.17.1. 5-(3-Fluoro-4-trifluoromethyl-phenyl)-[1,3,4]oxathiazol-2-one (26). The reaction mixture of 3-fluoro-4-trifluoromethylbenzamide **25** (2.82 g, 13.6 mmol), chlorocarbonylsulfenyl chloride (3.57 g, 27.2 mmol) in toluene (35 mL) was heated at 60 °C for 15 h and concentrated. CH_2Cl_2 was added and the mixture was filtered. The filtrate was concentrated and the product was purified by column chromatography (EtOAc/hexane) to provide 3.46 g (96%) of **26** as off-white solids: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, J = 8.2 Hz, 1H), 7.83 (d, J = 10.6 Hz, 1H), 7.77 (m, 1H).

5.1.17.2. 5-(3-Chloro-4-trifluoromethoxy-phenyl)-[1,3,4]-oxathiazol-2-one (32). Using **31** and following the procedure as in the preparation of **26** gave **32** (88%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, J = 2.0 Hz, 1H), 7.92 (dd, J = 8.7, 2.0 Hz, 1H), 7.45 (dd, J = 8.6, 1.1, 1H).

5.1.17.3. 5-(3,4-Dichloro-phenyl)-[1,3,4]oxathiazol-2-one (37). Using **36** and following the procedure as in the preparation of **26** gave **37** (94%) as an off-white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, J = 2.0 Hz, 1H), 7.80 (dd, J = 8.4, 2.0 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H).

5.1.17.4. 5-(2,4-Dichloro-phenyl)-[1,3,4]oxathiazol-2-one (42). Using **41** and following the procedure as in the preparation of **26** gave **42** (54%) as an off-white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, J = 8.5 Hz, 1H), 7.56 (d, J = 2.1 Hz, 1H), 7.39 (m, 1H).

5.1.17.5. 5-(3,4-Dimethyl-phenyl)-[1,3,4]oxathiazol-2-one (47). Using **46** and following the procedure as in the preparation of **26** gave **47** (47%) as a white solid:^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.69 (d, J = 7.9 Hz, 1H), 7.24 (d, J = 8.2 Hz, 1H), 2.33 (s, 6H).

5.1.17.6. 5-(3-Chloro-4-methyl-phenyl)-[1,3,4]oxathiazol-2-one (52). Using **51** and following the procedure as in the preparation of **26** gave **52** (57%) as a light brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 1.7 Hz, 1H), 7.75 (dd, J = 8.0, 1.8 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H).

5.1.18. General procedure for the synthesis of 27, 33, 38, 43, 48, and 53

5.1.18.1. 3-(3-Fluoro-4-trifluoromethyl-phenyl)-[1,2,4]-thiadiazole-5-carboxylic acid ethyl ester (27). A reaction mixture of **26** (480 mg, 1.81 mmol) and ethyl cyanoformate (722 mg, 7.29 mmol) in 1,2-dichlorobenzene (7 mL) was heated at 160 °C for 20 h. After cooling down to room temperature, the reaction mixture was purified by column chromatography to give 400 mg (69%) of **27** as a brown solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, J = 8.2 Hz, 1H), 8.22 (d, J = 11.2 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 4.57 (q, J = 7.1 Hz, 2H), 1.50 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 321 ($M+H^+$).

5.1.18.2. 3-(3-Chloro-4-trifluoromethoxy-phenyl)-[1,2,4]-thiadiazole-5-carboxylic acid ethyl ester (33). Using **32** and following the procedure as in the preparation of **27** gave **33** (36%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, J = 2.1 Hz, 1H), 8.31 (dd, J = 8.6, 2.1 Hz, 1H), 7.44 (dd, J = 8.6, 1.4 Hz, 1H), 4.56 (q, J = 7.1 Hz, 2H), 1.49 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 375 ($M+Na^+$).

5.1.18.3. 3-(3,4-Dichloro-phenyl)-[1,2,4]thiadiazole-5-carboxylic acid ethyl ester (38). Using **37** and following the procedure as in the preparation of **27** gave **38** (91%) as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (d, J = 2.0 Hz, 1H), 8.20 (dd, J = 8.4, 2.0 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 4.56 (q, J = 7.1 Hz, 2H), 1.49 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 303 ($M+H^+$).

5.1.18.4. 3-(2,4-Dichloro-phenyl)-[1,2,4]thiadiazole-5-carboxylic acid ethyl ester (43). Using **42** and following the procedure as in the preparation of **27** gave **43** (55%) as a beige solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 2.0 Hz, 1H), 7.38 (dd, J = 8.4, 2.0 Hz, 1H), 4.56 (q, J = 7.1 Hz, 2H), 1.48 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 303 ($M+H^+$).

5.1.18.5. 3-(3,4-Dimethyl-phenyl)-[1,2,4]thiadiazole-5-carboxylic acid ethyl ester (48). Using **47** and following the procedure as in the preparation of **27** gave **48** (47%) as a brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.09 (d, J = 7.9 Hz, 1H), 7.24 (m, 1H), 4.55 (q, J = 7.1 Hz, 2H), 2.35 (s, 3H), 2.33 (s, 3H), 1.48 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 263 ($M+H^+$).

5.1.18.6. 3-(3-Chloro-4-methyl-phenyl)-[1,2,4]thiadiazole-5-carboxylic acid ethyl ester (53). Using **52** and following the procedure as in the preparation of **27** gave **53** (87%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, J = 1.7 Hz, 1H), 8.15 (dd, J = 7.9, 1.7 Hz, 1H), 7.35 (d, J = 7.9 Hz, 1H), 4.55 (q, J = 7.1 Hz, 2H), 2.44 (s, 3H), 1.49 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 283 ($M+H^+$).

5.1.19. General procedure for the synthesis of 28, 34, 39, 44, 49, and 54

5.1.19.1. [3-(3-Fluoro-4-trifluoromethyl-phenyl)-[1,2,4]-thiadiazol-5-yl]-methanol (28). To a solution of **27** (212 mg, 0.662 mmol) in EtOH (10 mL) at room temperature was added $NaBH_4$ (64 mg, 1.7 mmol). After

stirring for 2 h, a few drops of water were added to quench excess of hydride. EtOH was evaporated, and the residue was partitioned between CH_2Cl_2 and water. The organic phase was dried and concentrated to provide 162 mg (88%) of **28** as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, J = 8.5 Hz, 1H), 8.13 (d, J = 11.6 Hz, 1H), 7.71 (m, 1H), 5.19 (s, 2H); MS (ES) m/z: 279 ($M+H^+$).

5.1.19.2. [3-(3-Chloro-4-trifluoromethoxy-phenyl)-[1,2,4]-thiadiazol-5-yl]-methanol (34). Using **33** and following the procedure as in the preparation of **28** gave **34** (93%) as a beige solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (d, J = 2.1 Hz, 1H), 8.21 (dd, J = 8.6, 2.1 Hz, 1H), 7.42 (dd, J = 8.6, 1.4 Hz, 1H), 5.18 (s, 2H).

5.1.19.3. [3-(3,4-Dichloro-phenyl)-[1,2,4]thiadiazol-5-yl]-methanol (39). Using **38** and following the procedure as in the preparation of **28** gave **39** (86%) as a yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (d, J = 1.9 Hz, 1H), 8.15 (m, 1H), 7.82 (d, J = 8.4, 1H), 4.99 (s, 2H).

5.1.19.4. [3-(2,4-Dichloro-phenyl)-[1,2,4]thiadiazol-5-yl]-methanol (44). Using **43** and following the procedure as in the preparation of **28** gave **44** (56%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, J = 8.4 Hz, 1H), 7.55 (d, J = 2.1 Hz, 1H), 7.36 (dd, J = 8.4, 2.1 Hz, 1H), 5.20 (s, 2H); MS (ES) m/z: 261 ($M+H^+$).

5.1.19.5. [3-(3,4-Dimethyl-phenyl)-[1,2,4]thiadiazol-5-yl]-methanol (49). Using **48** and following the procedure as in the preparation of **28** gave **49** (85%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (s, 1H), 8.00 (d, J = 7.8 Hz, 1H), 7.24 (d, J = 7.9 Hz, 1H), 5.17 (s, 2H), 2.62 (brs, 1H), 2.34 (s, 3H), 2.32 (s, 3H); MS (ES) m/z: 221 ($M+H^+$).

5.1.19.6. [3-(3-Chloro-4-methyl-phenyl)-[1,2,4]thiadiazol-5-yl]-methanol (54). Using **53** and following the procedure as in the preparation of **28** gave **54** (90%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 1.7 Hz, 1H), 8.06 (dd, J = 7.9, 1.7 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 5.17 (s, 2H), 2.44 (s, 3H); MS (ES) m/z: 241 ($M+H^+$).

5.1.20. General procedure for the synthesis of 30, 35, 40, 45, 50, and 55

5.1.20.1. 2-{4-[3-(3-Fluoro-4-trifluoromethyl-phenyl)-[1,2,4]thiadiazol-5-ylmethylsulfanyl]-2-methyl-phenoxy}-2-methyl-propionic acid (30). To a solution of **28** (684 mg, 2.46 mmol) in CH_2Cl_2 (10 mL) were added carbon tetrabromide (896 mg, 2.70 mmol) and triphenylphosphine (707 mg, 2.70 mmol). The mixture was stirred at 0 °C for 1 h and room temperature for 1 h, concentrated, and purified by column chromatography to give 554 mg (66%) of the bromide as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 11.5 Hz, 1H), 7.72 (t, J = 7.6 Hz, 1H), 4.82 (s, 2H); MS (ES) m/z: 343 ($M+H^+$). To a mixture of the bromide (75 mg, 0.22 mmol) and 2-(4-mercapto-2-methyl-phenoxy)-2-methyl-propionic acid ethyl ester **29** (52 mg, 0.21 mmol) in CH_3CN (1.5 mL) and DMF (0.1 mL) was added Cs_2CO_3 (100 mg, 0.31 mmol). After stirring at room temperature for 15 min, the mixture was concentrated. The residue was diluted with EtOAc, washed with water and brine, dried, concentrated, and column chromatographed to give 85 mg (79%) of 30-ethyl ester as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, J = 8.4 Hz, 1H), 8.07 (d, J = 11.3 Hz, 1H), 7.69 (t, J = 7.7 Hz, 1H), 7.26 (m, 1H), 7.14 (dd, J = 8.5, 2.5 Hz, 1H), 6.57 (d, J = 8.5 Hz, 1H), 4.40 (s, 2H), 4.20 (q, J = 7.1 Hz, 2H), 2.19 (s, 3H), 1.58 (s, 6H), 1.20 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 515 ($M+H^+$). A solution of 30-ethyl ester (82 mg, 0.16 mmol) in MeOH (1.0 mL) and THF (1.0 mL) was treated with 2 N NaOH (1.0 mL, 2.0 mmol) for 4 h and concentrated. The residue was diluted with EtOAc and water, and acidified with concentrated HCl. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic phases were washed with brine, dried, concentrated, and column chromatographed to provide 77 mg (99%) of **30** as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, J = 8.1 Hz, 1H), 8.06 (d, J = 11.3 Hz, 1H), 7.69 (t, J = 7.6 Hz, 1H), 7.28 (m, 1H), 7.17 (dd, J = 8.6, 2.5 Hz, 1H), 6.72 (d, J = 8.5 Hz, 1H), 4.42 (s, 2H), 2.20 (s, 3H), 1.61 (s, 6H); MS (ES) m/z: 487 ($M+H^+$). HRMS calcd for $C_{21}H_{18}F_4N_2O_3S_2$: 486.0695. Found: 486.0712.

5.1.20.2. 2-{4-[3-(3-Chloro-4-trifluoromethoxy-phenyl)-[1,2,4]thiadiazol-5-ylmethylsulfanyl]-2-methyl-phenoxy}-2-methyl-propionic acid (35). Using **34** and following the procedure as in the preparation of **30** gave **35** (20%) as a light yellow oily solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, J = 2.1 Hz, 1H), 8.16 (dd, J = 8.6, 2.1 Hz, 1H), 7.40 (dd, J = 8.6, 1.5 Hz, 1H), 7.28 (d, J = 2.0 Hz, 1H), 7.17 (dd, J = 8.4, 2.3 Hz, 1H), 6.72 (d, J = 8.5 Hz, 1H), 4.42 (s, 2H), 2.20 (s, 3H), 1.61 (s, 6H); MS (ES) m/z: 519 ($M+H^+$). HRMS calcd for $C_{21}H_{18}F_3ClN_2O_4S_2$: 518.0349. Found: 518.0365.

5.1.20.3. 2-{4-[3-(3,4-Dichloro-phenyl)-[1,2,4]thiadiazol-5-ylmethylsulfanyl]-2-methyl-phenoxy}-2-methyl-propionic acid (40). Using **39** and following the procedure as in the preparation of **30** gave **40** (46%) as a light yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, J = 2.0 Hz, 1H), 8.06 (dd, J = 8.4, 2.0 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 2.1 Hz, 1H), 7.17 (dd, J = 8.4, 2.4 Hz, 1H), 6.73 (d, J = 8.5 Hz, 1H), 4.41 (s, 2H), 2.20 (s, 3H), 1.61 (s, 6H); MS (ES) m/z: 469 ($M+H^+$). Anal. calcd for $C_{20}H_{18}Cl_2N_2O_3S_2 0.10H_2O$: C, 50.98; H, 3.89; N, 5.94. Found: C, 50.60; H, 3.53; N, 5.71.

5.1.20.4. 2-{4-[3-(2,4-Dichloro-phenyl)-[1,2,4]thiadiazol-5-ylmethylsulfanyl]-2-methyl-phenoxy}-2-methyl-propionic acid (45). Using **44** and following the procedure as in the preparation of **30** gave **45** (62%) as a light yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, J = 8.4 Hz, 1H), 7.52 (d, J = 2.0 Hz, 1H), 7.33 (dd, J = 8.4, 2.1 Hz, 1H), 7.28 (d, J = 2.2 Hz, 1H), 7.19 (dd, J = 8.4, 2.4 Hz, 1H), 6.73 (d, J = 8.5 Hz, 1H), 4.44 (s, 2H), 2.20 (s, 3H), 1.61 (s, 6H); MS (ES) m/z: 469 ($M+H^+$). Anal. calcd for $C_{20}H_{18}Cl_2N_2O_3S_2$:

C, 51.17; H, 3.87; N, 5.97. Found: C, 50.80; H, 3.53; N, 5.72.

5.1.20.5. 2-{4-[3-(3,4-Dimethyl-phenyl)-[1,2,4]thiadiazol-5-ylmethylsulfanyl]-2-methyl-phenoxy}-2-methyl-propionic acid (50). Using **49** and following the procedure as in the preparation of **30** gave **50** (67%) as a light yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.94 (dd, J = 7.8, 1.5 Hz, 1H), 7.28 (d, J = 1.9 Hz, 1H), 7.21 (d, J = 7.8 Hz, 1H), 7.17 (dd, J = 8.5, 2.2 Hz, 1H), 6.71 (d, J = 8.5 Hz, 1H), 4.42 (s, 2H), 2.32 (s, 3H), 2.31 (s, 3H), 2.19 (s, 3H), 1.59 (s, 6H); MS (ES) m/z: 429 ($M+H^+$). HRMS calcd for $C_{22}H_{24}N_2O_3S_2$: 428.1228. Found: 428.1215.

5.1.20.6. 2-{4-[3-(3-Chloro-4-methyl-phenyl)-[1,2,4]thiadiazol-5-ylmethylsulfanyl]-2-methyl-phenoxy}-2-methyl-propionic acid (55). Using **54** and following the procedure as in the preparation of **30** gave **55** (55%) as a light yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (d, J = 1.7 Hz, 1H), 8.01 (dd, J = 8.0, 1.7 Hz, 1H), 7.30 (d, J = 8.2 Hz, 1H), 7.27 (s, 1H), 7.17 (dd, J = 8.5, 2.2 Hz, 1H), 6.72 (d, J = 8.5 Hz, 1H), 4.41 (s, 2H), 2.42 (s, 3H), 2.19 (s, 3H), 1.59 (s, 6H). HRMS calcd for $C_{21}H_{21}ClN_2O_3S_2$: 448.0682. Found: 448.0713.

5.1.21. General procedure for the synthesis of 59 and 63

5.1.21.1. 5-(4-Chlorophenyl)-[1,2,4]thiadiazole-3-carboxylic acid ethyl ester (59). A mixture of **58** (1.77 g, 10.1 mmol) and 4-chlorobenzonitrile (6.97 g, 50.5 mmol) in 1,2-dichlorobenzene (10 mL) was heated at 160 °C for 4 days. After cooling down to room temperature, the reaction mixture was concentrated and the product was purified by column chromatography to provide 353 mg (13%) of **59** as light brown crystals: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 8.6 Hz, 2H), 7.51 (d, J = 8.6 Hz, 2H), 4.55 (q, J = 7.1 Hz, 2H), 1.49 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 269 ($M+H^+$).

5.1.21.2. 5-(3,4-Dichloro-phenyl)-[1,2,4]thiadiazole-3-carboxylic acid ethyl ester (63). Using 3,4-dichlorobenzonitrile and following the procedure as in the preparation of **59** gave **63** (31%) as an off-white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, J = 2.0 Hz, 1H), 7.85 (dd, J = 8.3, 2.1 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 4.55 (q, J = 7.1 Hz, 2H), 1.49 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 305 ($M+H^+$).

5.1.22. General procedure for the synthesis of 60 and 64

5.1.22.1. [5-(4-Chloro-phenyl)-[1,2,4]thiadiazol-3-yl]-methanol (60). Using **59** and following the procedure as in the preparation of **28** gave **60** (97%) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.6 Hz, 2H), 7.49 (d, J = 8.6 Hz, 2H), 4.98 (s, 2H); MS (ES) m/z: 227 ($M+H^+$).

5.1.22.2. [5-(3,4-Dichloro-phenyl)-[1,2,4]thiadiazol-3-yl]-methanol (64). Using **63** and following the procedure as in the preparation of **28** gave **64** (84%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (d, J = 2.1 Hz, 1H), 7.78 (dd, J = 8.4, 2.1 Hz, 1H), 7.59 (d, J = 8.4 Hz, 1H), 4.99 (s, 2H); MS (ES) m/z: 261 ($M+H^+$).

5.1.23. General procedure for the synthesis of 61 and 65

5.1.23.1. 3-Bromomethyl-5-(4-chloro-phenyl)-[1,2,4]thiadiazole (61). To a solution of **60** (558 mg, 2.46 mmol) in CH_2Cl_2 (10 mL) were added carbon tetrabromide (896 mg, 2.70 mmol) and triphenylphosphine (707 mg, 2.70 mmol). The reaction mixture was stirred at 0 °C for 1 h and room temperature for 1 h, concentrated, and purified by column chromatography to give 599 mg (84%) of the bromide **61** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 8.6 Hz, 2H), 7.49 (d, J = 8.6 Hz, 2H), 4.68 (s, 2H); MS (ES) m/z: 289 ($M+H^+$).

5.1.23.2. 3-Bromomethyl-5-(3,4-dichloro-phenyl)-[1,2,4]-thiadiazole (65). Using **64** and following the procedure as in the preparation of **61** gave **65** (83%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (d, J = 2.1 Hz, 1H), 7.71 (dd, J = 8.3, 2.1 Hz, 1H), 7.52 (d, J = 8.3 Hz, 1H), 4.61 (s, 2H); MS (ES) m/z: 349 ($M+Na^+$).

5.1.24. General procedure for the synthesis of 62 and 66

5.1.24.1. 2-{4-[5-(4-Chloro-phenyl)-[1,2,4]thiadiazol-3-ylmethylsulfanyl]-2-methyl-phenoxy}-2-methyl-propionic acid (62). Using **61** and following the procedure as in the preparation of **30** gave **62** (46%) as an oily solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (m, 2H), 7.47 (m, 2H), 7.27 (m, 1H), 7.18 (dd, J = 8.4, 2.3 Hz, 1H), 6.73 (d, J = 8.5 Hz, 1H), 4.34 (s, 2H), 2.19 (s, 3H), 1.60 (s, 6H); MS (ES) m/z: 435 ($M+H^+$). HRMS calcd for $C_{20}H_{19}ClN_2O_3S_2$: 434.0526. Found: 434.0618.

5.1.24.2. 2-{4-[5-(3,4-Dichloro-phenyl)-[1,2,4]thiadiazol-3-ylmethylsulfanyl]-2-methyl-phenoxy}-2-methyl-propionic acid (66). Using **65** and following the procedure as in the preparation of **30** gave **66** (68%) as an oily solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 2.0 Hz, 1H), 7.73 (dd, J = 8.3, 2.0 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.26 (m, 1H), 7.16 (m, 1H), 6.72 (d, J = 8.4 Hz, 1H), 4.34 (s, 2H), 2.20 (s, 3H), 1.60 (s, 6H); MS (ES) m/z: 469 ($M+H^+$). Anal. calcd for $C_{20}H_{18}Cl_2N_2O_3S_2$: C, 51.17; H, 3.87; N, 5.97. Found: C, 51.06; H, 3.68; N, 5.63.

5.1.25. General procedure for the synthesis of 67 and 72

5.1.25.1. [3-(4-Trifluoromethyl-phenyl)-[1,2,4]thiadiazol-5-yl]-acetic acid ethyl ester (67). A reaction mixture of **7** (0.995 g, 4.03 mmol) and ethyl cyanoacetate (1.8 g, 16.1 mmol) in 1,2-dichlorobenzene (18 mL) was heated at 160 °C for 20 h. After cooling down to room temperature, the reaction mixture was purified by column chromatography to give 153 mg (12%) of **67** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (d, J = 8.1 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H), 4.34 (q, J = 7.2 Hz, 2H), 4.27 (s, 2H), 1.37 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 317 ($M+H^+$).

5.1.25.2. [3-(3,4-Dichloro-phenyl)-[1,2,4]thiadiazol-5-yl]-acetic acid ethyl ester (72). Using **71** and following the procedure as in the preparation of **67** gave **72** (14%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (d, J = 1.9 Hz, 1H), 8.11 (dd, J = 8.4 Hz, 2.0 Hz,

1H), 7.53 (d, J = 8.4 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 4.25 (s, 2H), 1.36 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 317 ($M+H^+$).

5.1.26. General procedure for the synthesis of 68 and 73

5.1.26.1. 2-[3-(4-Trifluoromethyl-phenyl)-[1,2,4]thiadiazol-5-yl]-ethanol (68). To a solution of **67** (180 mg, 0.57 mmol) in EtOH-THF (8–2 mL) at room temperature was added $NaBH_4$ (136 mg, 3.6 mmol). After stirring for 20 h, a few drops of water were added to quench excess of hydride. The solvent was evaporated, and the residue was partitioned between CH_2Cl_2 and water. The organic phase was dried and concentrated to give the crude alcohol **68**: MS (ES) m/z: 275 ($M+H^+$).

5.1.26.2. 2-[3-(3,4-Dichloro-phenyl)-[1,2,4]thiadiazol-5-yl]-ethanol (73). Using **72** and following the procedure as in the preparation of **68** gave **73** (64%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (d, J = 2.0 Hz, 1H), 8.11 (dd, J = 8.4 Hz, 2.0 Hz, 1H), 4.11 (t, J = 5.6 Hz, 2H), 3.41 (t, J = 5.6 Hz, 2H).

5.1.27. General procedure for the synthesis of 69 and 74

5.1.27.1. 5-(2-Bromo-ethyl)-3-(4-trifluoromethyl-phenyl)-[1,2,4]thiadiazole (69). Using **68** and following the procedure as in the preparation of **61** gave **69** (44%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (d, J = 8.1 Hz, 2H), 7.74 (d, J = 8.2 Hz, 2H), 3.84–3.80 (m, 2H), 3.76–3.71 (m, 2H).

5.1.27.2. 5-(2-Bromo-ethyl)-3-(3,4-dichloro-phenyl)-[1,2,4]thiadiazole (74). Using **73** and following the procedure as in the preparation of **61** gave **74** (86%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (d, J = 2.0 Hz, 1H), 8.11 (dd, J = 8.4 Hz, 2.0 Hz, 1H), 3.83–3.78 (m, 2H), 3.73–3.69 (m, 2H).

5.1.28. General procedure for the synthesis of 70 and 75

5.1.28.1. 2-Methyl-2-(2-methyl-4-{2-[3-(4-trifluoromethyl-phenyl)-[1,2,4]thiadiazol-5-yl]-ethylsulfanyl}-phenoxy)-propionic acid (70). Using **69** and following the procedure as in the preparation of **30** gave **70** (40%) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.42 (d, J = 8.1 Hz, 2H), 7.78 (d, J = 8.2 Hz, 2H), 7.23 (d, J = 1.8 Hz, 1H), 7.17 (d, J = 8.3 Hz, 1H), 6.76 (d, J = 8.4 Hz, 1H), 3.43 (t, J = 6.1 Hz, 2H), 3.30 (m, 2H), 2.16 (s, 3H), 1.54 (s, 6H); MS (ES) m/z: 481 ($M-H^+$). HRMS calcd for $C_{21}H_{20}Cl_2N_2O_3S_2$: 482.0946. Found: 482.0952.

5.1.28.2. 2-(4-{2-[3-(3,4-Dichloro-phenyl)-[1,2,4]thiadiazol-5-yl]-ethylsulfanyl}-2-methyl phenoxy)-2-methyl-propionic acid (75). Using **74** and following the procedure as in the preparation of **30** gave **75** (35%) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.29 (d, J = 1.9 Hz, 1H), 8.07 (dd, J = 8.4, 1.9 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 1.8 Hz, 1H), 7.15 (dd, J = 8.3, 1.9 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 3.38 (t, J = 6.0 Hz, 2H), 3.31 (m, 2H), 2.15 (s, 3H), 1.56 (s, 6H); MS (ES) m/z: 483 ($M+H^+$). HRMS calcd for $C_{21}H_{20}Cl_2N_2O_3S_2$: 482.0292. Found: 482.0302.

5.1.29. General procedure for the synthesis of 79, 80, 81, 82, and 83

5.1.29.1. 2-{4-[3-(3-Fluoro-4-trifluoromethyl-phenyl)-[1,2,4]thiadiazol-5-ylmethoxy]-2-methyl-phenoxy}-2-methylpropionic acid (79). Using **28** and **78**, and following the procedure as in the preparation of **30** gave **79** (40%) as a colorless oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18–8.26 (m, 2H), 7.99–8.03 (t, J = 7.9 Hz, 1H), 7.00 (d, J = 3.0 Hz, 1H), 6.87–6.90 (m, 1H), 6.74 (d, J = 8.9 Hz, 1H), 5.66 (s, 2H), 2.17 (s, 3H), 1.46 (s, 6H); MS (ES) m/z: 471 ($M+H^+$); HRMS calcd for $C_{21}H_{18}F_4N_2O_4S$: 470.0923. Found: 470.0914.

5.1.29.2. 2-Methyl-2-{2-methyl-4-[3-(4-trifluoromethoxy-phenyl)-[1,2,4]thiadiazol-5-ylmethoxy]-phenoxy}-propionic acid (80). Using **34** and **78**, and following the procedure as in the preparation of **30** gave **80** (51%) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.47 (d, J = 2.0 Hz, 1H), 8.32 (dd, J = 8.6, 2.1 Hz, 1H), 7.58 (dd, J = 8.6, 1.4 Hz, 1H), 6.94 (s, 1H), 6.83–6.82 (m, 2H), 5.55 (s, 2H), 2.23 (s, 3H), 1.53 (s, 6H); MS (ES) m/z: 503 ($M+H^+$). Anal. calcd for $C_{21}H_{18}ClF_3N_2O_5S$: C, 50.16; H, 3.61; N, 5.57. Found: C, 50.16; H, 3.33; N, 5.43.

5.1.29.3. 2-{4-[3-(3,4-Dichloro-phenyl)-[1,2,4]thiadiazol-5-ylmethoxy]-2-methyl-phenoxy}-2-methyl-propionic acid (81). Using **39** and **78**, and following the procedure as in the preparation of **30** gave **81** (15%) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.40 (s, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.66 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 2.6 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 5.53 (s, 2H), 2.23 (s, 3H), 1.52 (s, 6H); MS (ES) m/z: 453 ($M+H^+$). HRMS calcd for $C_{20}H_{18}Cl_2N_2O_4S$: 452.0364. Found: 452.0385.

5.1.29.4. 2-{4-[3-(3,4-Dimethyl-phenyl)-[1,2,4]thiadiazol-5-ylmethoxy]-2-methyl-phenoxy}-2-methyl-propionic acid (82). Using **49** and **78**, and following the procedure as in the preparation of **30** gave **82** (17%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (s, 1H), 8.02 (d, J = 7.7 Hz, 1H), 7.23 (s, 1H), 6.85–6.91 (m, 2H), 6.75–6.79 (dd, J = 8.7, 3.2 Hz, 1H), 5.48 (s, 2H), 2.34 (d, J = 7.0 Hz, 6H), 2.26 (s, 3H), 1.57 (s, 6H); MS (ES) m/z: 413 ($M+H^+$); HRMS calcd for $C_{22}H_{24}N_2O_4S$: 413.1535. Found: 413.1533.

5.1.29.5. 2-{4-[3-(3-Chloro-4-methyl-phenyl)-[1,2,4]thiadiazol-5-ylmethoxy]-2-methyl-phenoxy}-2-methyl-propionic acid (83). Using **54** and **78**, and following the procedure as in the preparation of **30** gave **83** (26%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 6.72 (s, 1H), 6.57 (d, J = 7.9 Hz, 1H), 5.89 (d, J = 7.7 Hz, 1H), 5.40 (s, 1H), 5.26–5.33 (m, 2H), 4.0 (d, J = 1.5 Hz, 2H), 1.77 (s, 6H), 0.90 (s, 3H), 0.70 (s, 3H); MS (ES) m/z: 433 ($M+H^+$). Anal. calcd for $C_{21}H_{21}ClN_2O_4S$: C, 58.26; H, 4.89; N, 6.47. Found: C, 57.85; H, 4.86; N, 6.46.

5.1.30. 4-[3-(4-Trifluoromethyl-phenyl)-[1,2,4]thiadiazol-5-ylmethylsulfanyl]-phenol (85). To a solution of **84** (795 mg, 3.06 mmol) in CH_2Cl_2 (30 mL) at 0 °C were added methanesulfonyl chloride (518 mg, 4.52 mmol)

and triethylamine (617 mg, 6.11 mmol). The mixture was stirred at room temperature for 1 h and then partitioned between water and CH_2Cl_2 (80 mL). The organic layer was washed with brine, dried, concentrated, and column chromatographed (EtOAc/hexane) to provide 859 mg (83%) of the mesylate as a white solid. A mixture of the mesylate (210 mg, 0.621 mmol) and 4-mercaptophenol (113 mg, 0.897 mmol) in CH_3CN (8 mL) was degassed under N_2 for about 10 min. After the addition of Cs_2CO_3 (242 mg, 0.742 mmol), the mixture was stirred at room temperature for 40 min, concentrated, and purified by column chromatography (EtOAc/hexane) to give 228 mg (100%) of **85** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, J = 8.1 Hz, 2H), 7.71 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 8.7 Hz, 2H), 6.78 (d, J = 8.7 Hz, 2H), 4.97 (s, 1H), 4.40 (s, 2H); MS (ES) m/z: 369 ($M+H^+$).

5.1.31. 2-Methyl-2-{4-[3-(4-trifluoromethyl-phenyl)-[1,2,4]-thiadiazol-5-ylmethylsulfanyl]-phenoxy}-propionic acid (86). To a three-necked flask containing NaH (36 mg, 0.90 mmol; 60% in mineral oil) was added a solution of **85** (220 mg, 0.598 mmol) in THF. To the mixture at 40 °C was added *tert*-butyl 2-bromoisobutyrate (287 mg, 1.29 mmol). After heating at 70 °C for 2 h, more *tert*-butyl 2-bromoisobutyrate (215 mg, 0.970 mmol) was added and the heating was continued overnight. The reaction mixture was quenched with water (0.1 mL), concentrated, and chromatographed (EtOAc/hexane) to give 81 mg (27%) of 86-*tert*-butyl ester:^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, J = 8.2 Hz, 2H), 7.71 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.8 Hz, 2H), 6.78 (d, J = 8.7 Hz, 2H), 4.42 (s, 2H), 1.55 (s, 6H), 1.38 (s, 9H); MS (ES) m/z: 511 ($M+H^+$). The mixture of 86-*tert*-butyl ester (80 mg, 0.16 mmol) in CH_2Cl_2 (1.5 mL) and trifluoroacetic acid (0.5 mL) was stirred at room temperature for 1.5 h, concentrated, and column chromatographed (EtOAc/hexane, CH_2Cl_2/MeOH) to give 40 mg (56%) of **86** as a light yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, J = 8.0 Hz, 2H), 7.71 (d, J = 8.2 Hz, 2H), 7.37 (d, J = 8.8 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 4.45 (s, 2H), 1.59 (s, 6H); MS (ES) m/z: 453 ($M-H^+$). HRMS calcd for $C_{20}H_{17}F_3N_2O_3S_2$: 454.0633. Found: 454.0658.

5.1.32. 1-{4-[3-(3,4-Dichloro-phenyl)-[1,2,4]thiadiazol-5-ylmethylsulfanyl]-2-methyl-phenoxy}-cyclopentanecarboxylic acid (89). To a mixture of the bromide of **39** (**39A**) (58 mg, 0.18 mmol) and **88** (76 mg, 0.18 mmol) in THF (0.5 mL) at 0 °C was added 1.0 M tetrabutylammonium fluoride (0.18 mL, 0.18 mmol) in THF dropwise. After stirring at the same temperature for 15 min, the mixture was concentrated and the residue was purified by column chromatography to afford 88 mg (93%) of 89-methyl ester as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, J = 2.0 Hz, 1H), 8.07 (dd, J = 8.4, 2.0 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.26 (s, 1H), 7.13 (dd, J = 8.5, 2.4 Hz, 1H), 6.37 (d, J = 8.5 Hz, 1H), 4.38 (s, 2H), 3.70 (s, 3H), 2.28–2.12 (m, 4H), 2.18 (s, 3H), 1.81–1.76 (m, 4H); MS (ES) m/z: 509 ($M+H^+$). Using 89-methyl ester and following the same base hydrolysis procedure as in the preparation of **30** gave **89** (35%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, J = 2.0 Hz, 1H), 8.06 (dd, J = 8.4, 2.0 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.28–7.26 (m, 1H), 7.17 (dd, J = 8.4, 2.3 Hz, 1H), 6.50 (d, J = 8.5 Hz, 1H), 4.39 (s, 2H), 2.36–2.27 (m, 2H), 2.21–2.15 (m, 2H), 2.17 (s, 3H), 1.82–1.79 (m, 4H); MS (ES) m/z: 495 ($M+H^+$). HRMS calcd for $C_{22}H_{20}Cl_2N_2O_3S_2$: 494.0292. Found: 494.0311.

5.2. Biology

5.2.1. PPAR assays. All cell culture and co-transfection reagents were purchased from Invitrogen (Carlsbad, CA) with the exception of the Charcoal-treated FBS (Hyclone; Logan, UT). HEK293 cells were grown in DMEM/F-12 medium supplemented with 10%FBS and glutamine. The Steady-Glo Luciferase Assay Kit (Promega; Madison, WI) was used for measuring luciferase reporter activity. For a 175 cm^2 tissue culture flask, 100×10^5 of HEK293 cells were seeded in the growth medium and incubated at 37 °C in 5% CO_2 incubator until the cells were 80% confluent. The cells were then co-transfected with DNA constructs containing the ligand-binding domains of either PPARα, γ, or δ-Gal4 chimeric receptors and Gal4-luciferase reporter using DMRIE-C transfection reagent and OPTI-MEM reduced serum medium according to the manufacturer's instructions. On the following day, the DNA-containing medium was replaced with 30 mL of 5% Charcoal-treated FBS growth medium. After 6 h, the cells were re-plated at a density of 50,000 cells/well in 96-well plates and incubated overnight at 37 °C in a 5% CO_2 incubator. Cells were treated with 5 μL of compound or vehicle (0.1% DMSO) solution and incubated for 24 h at 37 °C in 5% CO_2 incubator. The next day, luciferase activity was measured with the Steady-Glo Luciferase Assay kit according to the manufacturer's instructions.

5.2.2. Induction of selected genes. Human peripheral blood lymphocytes were freshly prepared (ABS Inc., Wilmington, DE) and total RNA was isolated from the cells following manufacturer's instruction (Trizon method, Invitrogen Inc., CA). PCR primers and fluorescent probes (TaqMan probe) were designed using the software PrimerDesign (Applied Biosystems, Foster City, Ca). The sequences of the primers and TaqMan probe for PDHK4 (GenBank Accession No.: U54617) are 5′-TGCATTTTTGCGACAAGAATTG (forward), 5′-TTGGGTCGGGAGGATATCAA (reverse), and 5-FAM-CTGTGAGACTCGCCAACATTCTGAAGG A-TAMRA-3. The sequences of the primers and TaqMan probe for CPT1 (GenBank Accession No.: NM_001876) are 5′-CATTCCTTCC CATTCGTAGC (forward), 5′-AGCTGCACAA AGGCGTCTG (reverse), and 5-FAM-AGGAATCATC AAGAAATGTC GCACGAGC-TAMRA-3 (TaqMan). They were synthesized by Keystone Labs (Camarillo, CA). Reverse transcription (RT) was conducted following manufacturer's instruction using TaqMan Reverse Transcription Reagents (Applied BioSystems, CA). The RT reaction mixture was 20 μL containing 1× RT buffer, 5.5 mM $MgCl_2$, 0.5 mM dNTP, 2.5 μM of random hexamer, 1 U of RNase Inhibitor, 1 U of MultisScribe RT Enzyme, and 0.5 μg of total RNA. The reaction was carried

out at 48 °C for 30 min, then 95 °C for 5 min and finally at 4 °C. The reaction volume was extended into 62.5 μL and 5 μL of the RT (equivalent to 40 ng of total RNA) was used for PCR amplification. Each reaction was carried out in a total volume of 25 μL containing 5 μL of RT, 1× Master Mix (reaction buffer, dNTP, Polymerase, Applied Biosystems), 0.2 μM of forward, reverse primers, and TaqMan probe. The reaction was performed in a DNA Sequence Detector (Model 7900, Applied BioSystems) with the condition of 50 °C for 2 min, 94 °C for 10 min, followed by 40 cycles of 94 °C for 15 s and 59 °C for 1 min. For real time PCR quantitation, the measurement of human 18S ribosomal RNA was used as an internal control for normalization of measuring and loading errors. PCR data were collected by Ct value (the cycle number at which logarithmic PCR plots cross a calculated threshold line) according to the manufacturer's guidelines and the value was used to determine the Δ^{Ct} (Ct of the target gene minus the Ct of 18S ribosomal RNA controls). Relative mRNA level was calculated using the equation $2^{-\Delta\Delta Ct}$.

5.2.3. In vivo rat model. Male Sprague–Dawley rats weighing between 275 and 325 g at the start of treatment were used (ACE Animals, Inc., Boyertown, PA). All but six rats were fed an atherogenic (high cholesterol) diet (C13002, Research Diets, New Brunswick, NJ) for 6 days before starting treatment. The remaining six rats received normal chow (Purina 5001) and were also orally dosed with vehicle during the treatment period. The diets continued during the treatment period. The compound was formulated in 0.5% Methocel™, which was also the vehicle for the control animals and the animals were orally dosed at a volume of 10 mL/kg once daily for 8 days. On day 9, the animals were anesthetized with 70% CO_2/30% O_2 and blood was obtained from the retro-orbital sinus. Serum was prepared and the lipid parameters were measured using a COBAS analyzer (Roche Diagnostics). The animals were then sacrificed, the livers were removed (from six animals per group) and weighed.

5.2.4. In vivo hApoA1 and ob/ob mouse models. Male hAPOA1 transgenic mice and Female ob/ob mice (C57BL/6J-Lepob) were purchased from Jackson Labs (Bar Harbor, ME). Compound **40** in 0.5% methylcellulose suspension was dosed orally for 7 or 11 days, respectively. Serum levels of HDL-C, triglyceride, free fatty acids, and/or glucose were collected under fed conditions measured using a COBAS Mira Plus blood chemistry analyzer (Roche Diagnostic Systems, Indianapolis, IN). Statistical analysis was performed using the program Prism (Graphpad, Monrovia, CA) and with one-way analysis of variance and Dunnett's multiple comparison test.

5.2.5. Obese Beagle dog model. Female obese Beagle dogs (retired breeders) (Marshall Bioresources, North Rose, NY) were maintained on a regular canine diet and food was supplied once daily. Compound **40** in a dose range of 0.3–10 mg/kg was orally administered daily for continuous 7 days, approximately 3 h before feeding. Blood samples were collected 6 h after the last dose. Serum levels of triglycerides, total cholesterol, free fatty acids, AST, and ALT were measured by COBAS Mira Plus blood chemistry analyzer (Roche Diagnostic Systems, Indianapolis, IN).

5.2.6. Pharmacokinetic assay. Rats are normally dosed intravenously (iv) at levels of 1–3 mg/kg and by oral gavage at a level of 3–10 mg/kg with drug candidates. Drug compound is typically formulated for iv dosing as a solution in 10% w/v Solutol in 5% dextrose in sterile water vehicle (D5W). Drug compound is typically formulated for oral dosing as a uniform suspension in 0.5% methylcellulose vehicle. Blood samples (0.5 mL) are collected into heparinized tubes post-dose via orbital sinus puncture. Blood samples are centrifuged for cell removal, and precisely 200 μL of plasma supernatant is then transferred to a clean vial, placed on dry ice, and subsequently stored in a −70 °C freezer prior to analysis. Plasma samples are normally prepared as follows. Four hundred microliters of acetonitrile containing internal standard is added to 200 μL of plasma to precipitate proteins. Samples are centrifuged at 5000*g* for 3 min and supernatant removed for analysis by LC–MS–MS. Calibration standards are prepared by adding appropriate volumes of stock solution directly into plasma and treated identically to collected plasma samples. Calibration standards are typically prepared in the range of 0.1–10 mM for quantitation. LC–MS–MS analysis was performed using either multiple reaction or selected ion monitoring for detection of characteristic ions for each drug candidate and internal standard used is Propranolol.

5.3. Computational details

PPARα/az242 (PDB Accession 1I7G)[33] and PPARδ/gw2433 (PDB Accession 1GWX)[34] were used as protein coordinates in the Glide docking studies. All ligand initial 3D coordinates were generated with an in-house implementation of a Stochastic Proximity Embedding (SPE) algorithm,[35] followed by minimization using the MMFF94s force-field.[36–39] Explicit hydrogens were added to the proteins using the 'all-atom with no lone pair treatment' followed by restrained minimization, as implemented in PPREP utility of Glide4.5.[27,28] Various Coulombic and van der Waals grids were generated with, and without, hydrogen bond constraints to Y473δ and Y464α. Compound **21** was then allowed to flexibly dock into all receptors using standard precision (SP) or extra precision (XP) Glide. GlideScore, the default internal docking scoring function in Glide, evaluated the interaction energies of ligand with the Coulombic and van der Waals grids. Then the best scoring poses are minimized using the OPLS-AA forcefield[40] on precomputed van der Waals and electrostatic grids.

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