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Synthesis and Biological Evaluation of 2-Heteroarylthioalkanoic Acid Analogues of Clofibric Acid as Peroxisome Proliferator-Activated Receptor α Agonists

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A series of 2-heteroarylthioalkanoic acids were synthesized through systematic structural modifications of clofibric acid and evaluated for human peroxisome proliferator-activated receptor α (PPARα) transactivation activity, with the aim of obtaining new hypolipidemic compounds. Some thiophene and benzothiazole derivatives showing a good activation of the receptor α were screened for activity against the PPARγ isoform. The gene induction of selected compounds was also investigated in the human hepatoma cell line.

Introduction

The nuclear peroxisome proliferator-activated receptors (PPARs[a]) are ligand-dependent transcription factors belonging to the nuclear receptor superfamily.[1] Upon ligand binding, PPARs heterodimerize with the retinoid X receptor (RXR) and regulate target gene transcription at specific consensus DNA sequences, the peroxisome proliferator response elements (PPRE).[2] Three subtypes of PPARs (PPARα, PPARγ, and PPARδ) have been identified to date. Their endogenous ligands include fatty acids and eicosanoids.[3] PPARα is predominantly expressed in liver, where it plays a critical role in lipid homeostasis by regulating fatty acid binding, uptake, and oxidation.[4] It is also expressed in a variety of cell types, including smooth muscle cells, endothelial cells, and macrophages, playing a pivotal role in processes such as atherosclerosis and inflammation.[5]

Several studies have analyzed the overall contribution of PPARα to cardiovascular disease, and a large number of synthetic activators with atheroprotective properties in humans have been described.[6] Fibrates are the only marketed PPARα agonists that are effective in lowering elevated serum triglyceride levels and moderately raising high-density lipoproteins (HDL). Their chemical structures are characterized by the presence of the 2-phenoxy-2-methylpropanoic moiety. Fibrates have weak affinities to both rodent and human cloned receptors, with extremely poor subtype selectivity,[7] and necessitate use of high micromolar concentrations to activate human PPARα (30−55 μM). Recent advances in nuclear receptor ligand discovery have led to the identification of a second generation PPARα agonists with affinities for activation in the nanomolar range compared to the micromolar range for the fibrates. Examples are the ureido-based fibric acid GW7647[8] (**1**), the phenylpropanoic acid derivative KCL1998001079[9] (**2**), the fibrate LY518674[10] (**3**), the 2,3-dihydrobenzofuran-2-carboxylic acids[11] **4**, which are constrained analogues of fibric acid, the indaneureidothioisobutyric acids[12] **5**, and the 1,3-dioxane-2-carboxylic acids[13] **6** (Figure 1).

In this study, we aimed to optimize the PPARα binding profile and the in vitro efficacy of classical fibrates by synthesizing a series of new compounds derived from chemical modifications of clofibric acid (**7**), the active metabolite of clofibrate (Figure 2). The clofibric acid moiety was changed by substituting the oxygen with sulfur, thus leading to thioalkanoic acids. A previous example of this modification is the potent and selective PPARα agonist **1**.[8] Moreover, the phenyl ring was replaced with the heterocyclic scaffolds thiophene, pyridine, and benzothiazole, with different substitution patterns. In some compounds a chiral center was introduced in α-position to the carboxylic group. For reference compounds in the pharmacological evaluations, we selected compounds **7** and **1**. On the basis of in vitro PPARα transactivation assay results, some compounds were also tested on PPARγ and were selected for the in vitro analysis of target gene expression.

Chemistry

Compounds **8**, **9a**,**b**, and **12a**−**u** (Table 1) were easily obtained in good yields by standard esterification procedures followed by hydrolysis. We have previously described the synthesis of **8**, **9a**,**b**, and **12a**,**b**, which were found to have interesting antiplatelet properties.[14] Esters **11c**−**u** were obtained

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[a] Abbreviations: PPARs, peroxisome proliferator-activated receptors; RXR, retinoid X receptor; PPRE, peroxisome proliferator response elements; HDL, high-density lipoproteins; CPT1A, carnitine palmitoyl transferase 1A; HepG2, human hepatocellular liver carcinoma cell line; RTqPCR, real-time quantitative polymerase chain reaction; HEK293, human embryonic kidney 293 cell line; FBS-GOLD, fetal bovine serum "GOLD".

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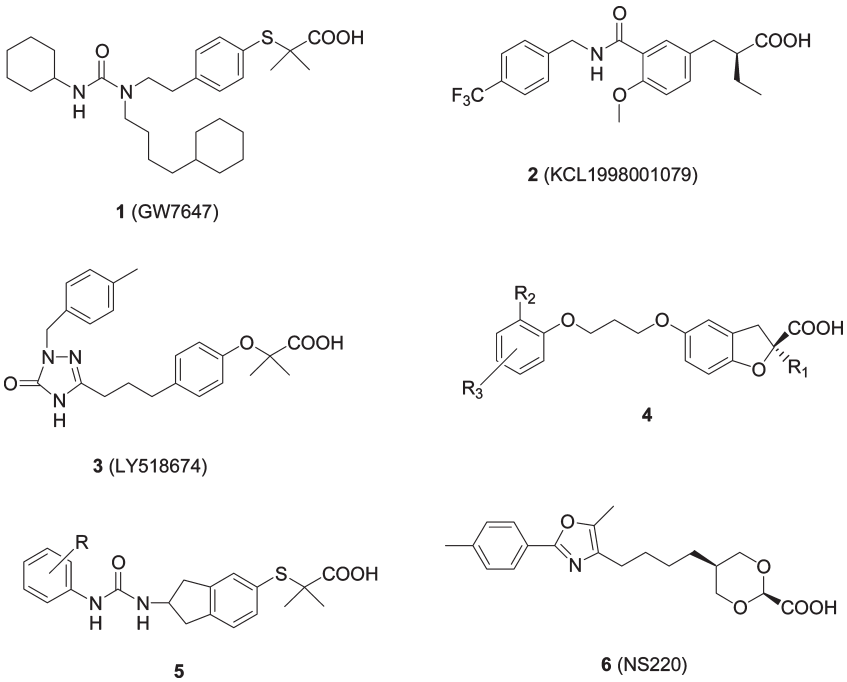


Figure 1. Selective PPARα agonists.

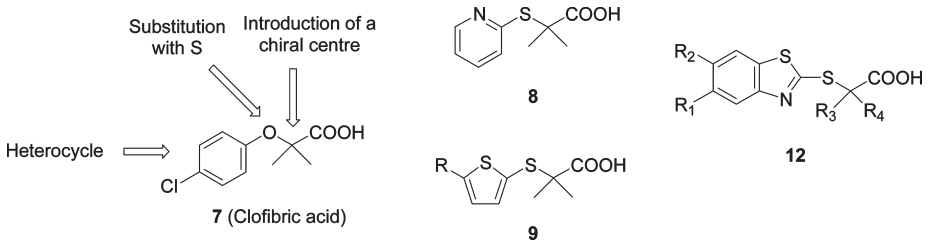


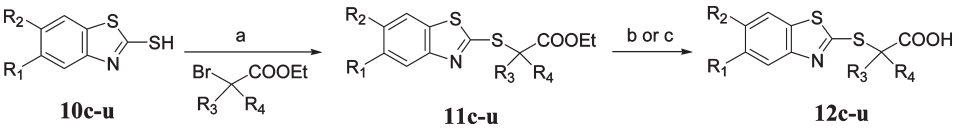
Figure 2. Structural modifications of clofibric acid.

by S_N2 reaction of 2-mercaptoheterocycles (**10c**−**u**) with proper 2-bromoesters, in the presence of sodium in absolute ethanol at reflux. The basic hydrolysis by 1 N NaOH of **11c**−**u** gave the acids **12c**−**u**. When not commercially available, substituted 2-mercaptobenzothiazoles were synthesized according to well established procedures.[15] The *rac*-**12l** and *rac*-**12o** acids were also synthesized in enantiomerically pure form. In this case, we started from optically active (*R*)- and (*S*)-2-bromoacids, esterified with ethanol, toluene, and concentrated sulfuric acid in a Dean−Stark apparatus to obtain the corresponding bromoesters, which were used for S_N2 reaction with 2-mercaptoheterocycles **10l** and **10o** to give (*R*)- and (*S*)-**11l** and (*R*)- and (*S*)-11o. Finally, the optically pure (*R*)- and (*S*)-**12l** and (*R*)- and (*S*)-**12o** acids were obtained by hydrolysis with 6 N HCl (Scheme 1).

Results and Discussion

With the aim of identifying new compounds able to activate the PPARα receptor, **8**, **9a**,**b**, and *rac*-**12a**−**u** were evaluated for human PPARα functional activity by a cell-based transactivation assay in eukaryotic cells,[16] a powerful and widely used method whose good correlation with in vivo activity is generally accepted. First, we evaluated the activity of new compounds at a concentration of 150 μM, with **7** (150 μM) and **1** (1 μM) as reference compounds; results are expressed as fold activation. Then the compounds with the best activity were selected for the determination of their EC_{50} values. Results are shown in Table 1. Compounds **8**, with pyridine, and **9a**, with thiophene, were found inactive on PPARα, while the introduction of a chlorine atom on the 5-position of thiophene (**9b**) resulted in a significant activation of the receptor (EC_{50} = 1.8 μM). In the series of (2-benzothiazolyl)thioisobutanoic acids **12a**−**k**, structural modifications focused on substitution of the benzothiazolic ring. While unsubstituted derivative **12a** proved inactive, the overall effect of the introduction of substituents in the 5 and 6 positions improved PPARα agonistic activity except for **12e** and **12i**, which were inactive. Of note, among these compounds was **12c**, the 5-bromine derivative, which is characterized by an EC_{50} value of 2.5 μM and is thus 10−20 times more effective than other compounds of the same series. As reported in previous works, chiral aryloxyacid analogues of clofibric acid were found to exhibit antilipidemic and antiplatelet activity. It has been clearly demonstrated that pharmacological and toxic effects are influenced by chirality.[17] Recent screening tests of other chiral analogues of clofibric acid have indicated that some are potent PPARα/γ agonists.[18] On the basis of these findings, we focused on introducing a chiral center in the α-position of some of the above-described heterocyclic analogues of clofibric acid (*rac*-**12l**−**u**, R_3 = H) and the new compounds were preliminarily tested in racemic form. The monomethylated derivatives *rac*-**12l**−**q** did not show significant changes of PPARα activity in comparison with dimethyl

Scheme 1[a]



[a] Reagents and conditions: (a) Na, absolute EtOH, reflux, N_2; (b) 1N NaOH, room temp; (c) 6 N HCl, 60 °C for enantiomeric compounds.

Table 1. In Vitro Human PPARα Transactivation of Test and Reference Compounds

compd	R_1	R_2	R_3	R_4	FA[a]	EC_{50} (μM)[b]
8					na[c]	
9a					na[c]	
9b					2.8 ± 0.2	1.8 ± 0.1
12a	H	H	Me	Me	na[c]	
12b	Cl	H	Me	Me	1.6 ± 0.1	35.9 ± 2.7
12c	Br	H	Me	Me	4.5 ± 1.0	2.5 ± 0.1
12d	CN	H	Me	Me	1.9 ± 0.6	46.3 ± 3.6
12e	CF_3	H	Me	Me	na[c]	
12f	OMe	H	Me	Me	2.0 ± 0.2	45.8 ± 3.9
12g	H	Cl	Me	Me	1.7 ± 0.1	38.9 ± 2.5
12h	H	Br	Me	Me	2.6 ± 0.4	45.8 ± 4.0
12i	H	CN	Me	Me	na[c]	
12j	H	CF_3	Me	Me	1.7 ± 0.2	36.1 ± 2.9
12k	H	OEt	Me	Me	3.3 ± 0.8	69.3 ± 5.4
rac-**12l**	Cl	H	H	Me	3.4 ± 0.2	62.5 ± 5.9
rac -**12m**	Br	H	H	Me	3.2 ± 0.6	9.3 ± 0.9
rac -**12n**	CN	H	H	Me	na[c]	
rac -**12o**	OMe	H	H	Me	2.9 ± 0.2	33.3 ± 4.5
rac -**12p**	H	Br	H	Me	1.9 ± 0.9	84.3 ± 6.4
rac -**12q**	H	OEt	H	Me	2.6 ± 0.2	9.8 ± 1.3
rac -**12r**	Cl	H	H	*n*-Pro	2.7 ± 0.6	9.3 ± 0.9
rac -**12s**	H	OEt	H	*n*-Pro	4.7 ± 0.1	14.8 ± 1.2
rac -**12t**	Cl	H	H	*n*-Hex	na[c]	
rac -**12u**	Cl	H	H	Ph	1.6 ± 0.1	14.3 ± 1.2
7					1.6 ± 0.2	55.0 ± 3.9
1					2.8 ± 0.5	0.2 ± 0.02

[a] FA: fold activation. Compounds were tested at least three separate experiments at 150 μM. Only **1** was tested at 1 μM. The results are expressed with ±SEM. [b] Compounds were tested in at least three separate experiments in at five concentrations ranging from 1 to 150 μM. The results are expressed with ±SEM. [c] Not active.

derivatives. Among them, only *rac*-**12m** (R_1 = Br) and *rac*-**12q** (R_2 = OEt) exhibited good values of EC_{50} (9.3 and 9.8 μM, respectively). Further structural modifications were carried out in the α-position to the carboxylic function in order to investigate the influence of more hydrophobic and sterically hindered groups on PPARα agonistic activity. Compounds with a *n*-propylic chain (*rac*-**12r**,**s**) or a phenyl group (*rac*-**12u**) demonstrated an increase in agonistic activity over their R_4-methyl-substituted analogues (**12k** and **12l**), while the further elongation of the substituent (*n*-hexylic chain, rac-**12t**) caused loss of activity. The transactivation studies reported in Table 1 show that the position, the size, and the electronic effect of R_1 and R_2 groups on benzothiazolic ring did not seem to correlate with activity. Indeed, there were no remarkable differences when varying the position and dimensions of substituents and when changing electron-withdrawing with electron-donor groups. Also, the introduction of a chiral center in α-position to the carboxylic moiety did not improve the transactivation activity except when using *n*-propyl and phenyl groups as substituents.

Considering the high degree of stereoselectivity generally displayed by PPAR ligands,[19] we were also interested in the biological evaluation of some optically active derivatives.

Table 2. In Vitro Human PPARα Transactivation of Racemic and Optically Active **12l** and **12o**

compd	R_1	R_2	R_3	R_4	FA[a]	EC_{50} (μM)[b]
rac-**12l**	Cl	H	H	Me	3.4 ± 0.2	62.5 ± 5.9
(*R*)-**12l**	Cl	H	H	Me	4.5 ± 0.8	73.5 ± 6.8
(*S*)-**12l**	Cl	H	H	Me	2.6 ± 1.6	55.9 ± 5.2
rac-**12o**	OMe	H	H	Me	2.9 ± 0.2	33.3 ± 4.5
(*R*)-**12o**	OMe	H	H	Me	2.3 ± 0.3	57.3 ± 4.7
(*S*)-**12o**	OMe	H	H	Me	2.9 ± 0.3	1.9 ± 0.1
7					1.6 ± 0.2	55.0 ± 3.9
1					2.8 ± 0.5	0.2 ± 0.02

[a] FA: fold activation. Compounds were tested at least three separate experiments at 150 μM. Only **1** was tested at 1 μM. The results are expressed with ±SEM. [b] Compounds were tested in at least three separate experiments in at five concentrations ranging from 1 to 150 μM. The results are expressed with ±SEM.

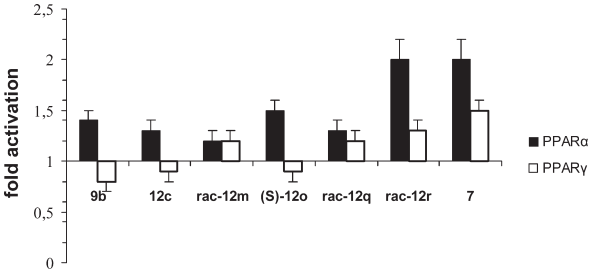


Figure 3. Selectivity PPAR α/γ.

Enantiomerically pure (*R*)- and (*S*)-**12l** and (*R*)- and (*S*)-**12o** acids were synthesized and tested. Data in Table 2 confirm the greater activity of (*S*)-isomers, in agreement with previous studies.[19] In particular, a clear stereopreference was found with (*S*)-**12o**, which exhibited more potent transactivation activity (EC_{50} = 1.9 μM) than the antipodal (*R*)-isomer (EC_{50} = 57.3 μM).

The fibrate analogues described in this work have, like the most selective PPARα activators, an aromatic system connected to an acidic headgroup. Although they are less potent than the compounds with a long hydrophobic tail linked to the aromatic scaffold, better values of EC_{50} than the classical fibrates were observed.

All compounds showing good activation of PPARα receptor (EC_{50} < 10 μM) were preliminarily tested on PPARγ in order to explore a possible selective activation of these receptors. We performed the transactivation assay on both PPARα and PPARγ isoforms, testing each compound at a concentration of PPARα EC_{50}. By comparing fold activation values, we observed the preferential activation of α-isoform for compounds **9b**, **12c**, and (*S*)-**12o** (Figure 3). This evaluation is only a qualitative overview; however, is noteworthy that best selectivity profiles PPARα/γ were obtained for compounds more active on PPARα (EC_{50} values within 3 μM).

The gene carnitine palmitoyl transferase 1A (CPT1A) has been previously shown to be induced in human hepatocytes and hepatoma cells in response to PPARα agonists; the investigation of CPT1A expression is a well established in

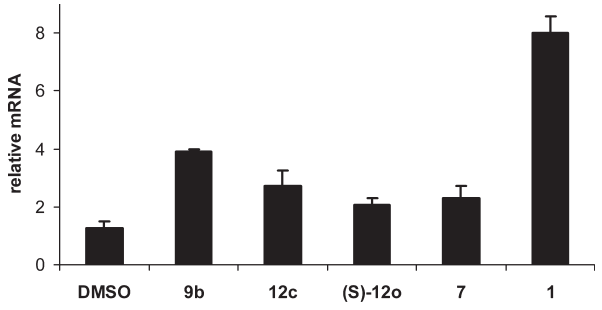


Figure 4. CPT1A expression in HepG2 following treatment. Cells were treated with vehicle (DMSO), **1**, **7**, **9b**, **12c**, and (*S*)-**12o** at 150 μM for 48 h. RTqPCR was performed to measure CPT1A mRNA levels. Values shown represent the mean ± SEM of four independent determinations performed in duplicate. Cyclophilin was used as reference gene, and values were normalized to data obtained from vehicle treated cells.

vitro model to study PPARα activation.[20] On the basis of the transactivation assay results, we selected **9b**, **12c**, and (*S*)-**12o** for the in vitro analysis of CPT1A expression in human hepatocellular liver carcinoma cell line (HepG2) by using real-time quantitative polymerase chain reaction (RTqPCR). As shown in Figure 4, a pronounced increase of gene expression of about 8-fold was detected when cells were treated for 48 h with reference compound **1**, while CPT1A mRNA levels were doubled with control **7**, according to the fibrate activity.[20] Treatment with **9b** increased the expression of gene by 2-fold, while compounds **12c** and (*S*)-**12o** displayed mRNA levels similar to control **7**.

Conclusions

A new series of clofibric acid analogues was synthesized and in vitro evaluated for human PPARα activity by transactivation assay. Overall, the potencies of some newly designed agonists were slightly higher than those of typical fibrates, such as clofibrate. The best findings were obtained with compounds **9b**, **12c**, and (*S*)-**12o**, showing EC_{50} values within 3 μM. Selectivity experiments on the PPARγ isoform also demonstrated their preferential activation of α-isoform. Also, the effects of gene induction were investigated by the analysis of CPT1A expression in the HepG2 cell line: compound **9b** was remarkably twice as effective as control **7**, making it a prime candidate as a new lead compound for the design of more potent and selective PPARα agonist analogues of fibrates.

Experimental Section

Chemistry. General. Melting points were determined on a Buchi B-540 apparatus and are uncorrected. Infrared spectra (IR) were recorded on a FT-IR 1600 Perkin-Elmer spectrometer. NMR spectra were run at 300 MHz on a Varian instrument; chemical shifts (δ) are reported in ppm. Microanalyses were carried out with an Eurovector Euro EA 3000 model analyzer, and the analytical results were within 0.4% of the theoretical values. Commercial reagents were used as received from Aldrich or Fluka. The optically active acids (*R*)- and (*S*)-**12l** and **12o** had enantiomeric excesses (ee) greater than 98% determined by using an HPLC device (Waters Association, Milford, MA) equipped with a Chiralpack AD or AI chiral column (25 cm × 4.6 mm) (Daicel Chemical Industries Ltd., Japan) connected to an UV detector (254 nm): mobile phase, *n*-Hex/EtOH = 95/5 + 0.05% TFA; flow rate, 1.0 mL/min.

Compounds **8**, **9a**,**b**, **11a**,**b**, and **12a**,**b** have been previously described.[14,15]

General Procedure for the Preparation of Esters 11c−k and *rac*-11l−u. The heterocyclic thiol (3.0 mmol) and ethyl 2-bromoalkanoate (3.0 mmol), both dissolved in absolute EtOH (10 mL), were added to a solution of sodium (69.0 mg, 3.0 mmol) in absolute EtOH (10 mL) under nitrogen atmosphere. After the mixture was stirred for 2−5 h at reflux, the solvent was removed under reduced pressure. The residue was poured into water (20 mL) and extracted with diethyl ether (3 × 20 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent cyclohexane/ethyl acetate, 95:5).

Ethyl 2-[(5-Bromo-1,3-benzothiazol-2-yl)thio]-2-methylpropanoate (11c). Orange-yellow oil, 47% yield. IR (KBr) 1728 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.21 (t, 3H, J = 7.2 Hz, CH_3CH_2), 1.75 (s, 6H, $C(CH_3)_2$), 4.20 (q, 2H, J = 7.2 Hz, CH_2CH_3), 7.41−7.45 (dd, 1H, J = 8.7 Hz, J = 2.1 Hz, CH Ar), 7.62 (d, 1H, J = 8.7 Hz, CH Ar), 8.04 (d, 1H, J = 2.1 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3CH_2), 26.6 ($C(CH_3)_2$), 54.5 ($C(CH_3)_2$), 62.1 (CH_2CH_3), 119.9 (C Ar), 122.2, 125.3, and 128.1 (CH Ar), 135.0 and 154.6 (C Ar), 164.9 (=C−S), 173.3 (C=O). Anal. ($C_{12}H_{14}BrNO_2S_2$) C, H, N.

Ethyl 2-[(5-Cyano-1,3-benzothiazol-2-yl)thio]-2-methylpropanoate (11d). Yellowish oil, 72% yield. IR (KBr) 2229, 1733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (t, 3H, J = 7.2 Hz, CH_3CH_2), 1.78 (s, 6H, $C(CH_3)_2$), 4.21 (q, 2H, J = 7.2 Hz, CH_2CH_3), 7.53−7.56 (dd, 1H, J = 8.1 Hz, J = 1.5 Hz, CH Ar), 7.86 (d, 1H, J = 8.1 Hz, CH Ar), 8.13 (d, 1H, J = 1.5 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.3 (CH_3CH_2), 26.6 ($C(CH_3)_2$), 54.9 ($C(CH_3)_2$), 62.2 (CH_2CH_3), 110.0 (C Ar), 118.93 (CN), 122.2, 126.0, and 127.3 (CH Ar), 140.9 and 153.0 (C Ar), 166.9 (=C−S), 173.1 (C=O). Anal. ($C_{14}H_{14}N_2O_2S_2$) C, H, N.

Ethyl 2-[(5-Trifluoromethyl-1,3-benzothiazol-2-yl)thio]-2-methylpropanoate (11e). Yellowish oil, 62% yield. IR (KBr) 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.21 (t, 3H, J = 7.2 Hz, CH_3CH_2), 1.78 (s, 6H, $C(CH_3)_2$), 4.21 (q, 2H, J = 7.2 Hz, CH_2CH_3), 7.56 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz, CH Ar), 7.87 (d, 1H, J = 8.4 Hz, CH Ar), 8.13 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3CH_2), 26.6 ($C(CH_3)_2$), 54.7 ($C(CH_3)_2$), 62.2 (CH_2CH_3), 119.4, 121.3, and 121.7 (CH Ar), 128.8 (CF_3), 129.2, 139.1, 153.1, and 165.8 (C Ar), 173.2 (C=O). Anal. ($C_{14}H_{14}F_3NO_2S_2$) C, H, N.

Ethyl 2-[(5-Methoxy-1,3-benzothiazol-2-yl)thio]-2-methylpropanoate (11f). Yellowish oil, 42% yield. IR (KBr) 1729 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (t, 3H, J = 7.2 Hz, CH_3CH_2), 1.74 (s, 6H, $C(CH_3)_2$), 3.87 (s, 3H, OCH_3), 4.19 (q, 2H, J = 7.2 Hz, CH_2CH_3), 6.97−7.01 (dd, 1H, J = 8.7 Hz, J = 2.7 Hz, CH Ar), 7.42 (d, 1H, J = 2.7 Hz, CH Ar), 7.62 (d, 1H, J = 8.7 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3CH_2), 26.6 ($C(CH_3)_2$), 54.1 ($C(CH_3)_2$), 55.8 (OCH_3), 62.0 (CH_2CH_3), 105.2, 115.4, and 126.4 (CH Ar), 128.5, 154.8, and 159.1 (C Ar), 163.0 (=C−S), 173.5 (C=O). Anal. ($C_{14}H_{17}NO_3S_2$) C, H, N.

Ethyl 2-[(6-Chloro-1,3-benzothiazol-2-yl)thio]-2-methylpropanoate (11g). Yellowish oil, 46% yield. IR (KBr) 1729 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.19 (t, 3H, J = 7.2 Hz, CH_3CH_2), 1.74 (s, 6H, $C(CH_3)_2$), 4.19 (q, 2H, J = 7.2 Hz, CH_2CH_3), 7.38 (dd, 1H, J = 9.0 Hz, J = 1.8 Hz, CH Ar), 7.75 (d, 1H, J = 1.8 Hz, CH Ar), 7.80 (d, 1H, J = 9.0 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3CH_2), 26.6 ($C(CH_3)_2$), 54.4 ($C(CH_3)_2$), 62.1 (CH_2CH_3), 120.8, 123.2, and 127.1 (CH Ar), 131.1, 137.6, 152.0, and 163.2 (C Ar), 173.3 (C=O). Anal. ($C_{13}H_{14}ClNO_2S_2$) C, H, N.

Ethyl 2-[(6-Bromo-1,3-benzothiazol-2-yl)thio]-2-methylpropanoate (11h). Yellowish oil, 63% yield. IR (KBr) 1741 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.19 (t, 3H, J = 7.2 Hz, CH_3CH_2), 1.74 (s, 6H, $C(CH_3)_2$), 4.19 (q, 2H, J = 7.2 Hz, CH_2CH_3), 7.52 (dd, 1H, J = 8.7 Hz, J = 2.1 Hz, CH Ar), 7.74 (d, 1H, J = 8.7 Hz, CH Ar), 7.90 (d, 1H, J = 2.1 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3CH_2), 26.6 ($C(CH_3)_2$), 54.4 ($C(CH_3)_2$), 62.1 (CH_2CH_3), 118.7 (C Ar),123.6, 123.7, and 129.8 (CH Ar), 138.0, 152.3, and 163.4 (C Ar), 173.3 (C=O). Anal. ($C_{13}H_{14}BrNO_2S_2$) C, H, N.

Ethyl 2-[(6-Cyano-1,3-benzothiazol-2-yl)thio]-2-methylpropanoate (11i). White solid, 47% yield. IR (KBr) 2225, 1720 cm^{-1}; mp

88−89 °C; ^{1}H NMR ($CDCl_3$) δ 1.19 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.79 (s, 6H, C(CH_3)$_2$), 4.20 (q, 2H, J = 7.2 Hz, CH$_3$CH_2CH$_3$), 7.66 (dd, 1H, J = 8.4 Hz, J = 1.5 Hz, CH Ar), 7.90 (d, 1H, J = 8.4 Hz, CH Ar), 8.08 (d, 1H, J = 1.5 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3CH$_2$), 26.6 (C(CH_3)$_2$), 54.9 (C(CH$_3$)$_2$), 62.2 (CH$_2$CH$_3$), 108.1 (C Ar), 118.9 (CN), 122.7, 125.7, and 129.6 (CH Ar), 136.3, 155.7, and 169.0 (C Ar), 173.1 (C=O). Anal. ($C_{14}H_{14}N_2O_2S_2$) C, H, N.

Ethyl 2-[(6-Trifluoromethyl-1,3-benzothiazol-2-yl)thio]-2-methylpropanoate (11j). Yellowish oil, 71% yield. IR (KBr) 1734 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.78 (s, 6H, C(CH_3)$_2$), 4.20 (q, 2H, J = 7.2 Hz, CH$_2$CH$_3$), 7.65 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz, CH Ar), 7.94 (d, 1H, J = 8.4 Hz, CH Ar), 8.06 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3CH$_2$), 26.6 (C(CH_3)$_2$), 54.7 (C(CH$_3$)$_2$), 62.2 (CH$_2$CH$_3$), 118.8, 122.5, and 123.3 (CH Ar), 126.1 (CF_3), 127.2, 136.1, 155.8, and 167.0 (C Ar), 173.2 (C=O). Anal. ($C_{14}H_{14}F_3NO_2S_2$) C, H, N.

Ethyl 2-[(6-Ethoxy-1,3-benzothiazol-2-yl)thio]-2-methylpropanoate (11k). Yellowish solid, 48% yield. IR (KBr) 1727 cm^{-1}; mp 77−78 °C; ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, J = 7.2 Hz, CH_3CH$_2$O), 1.43 (t, 3H, J = 7.2 Hz, CH_3CH$_2$OAr), 1.70 (s, 6H, C(CH_3)$_2$), 4.06 (q, 2H, J = 7.2 Hz, OCH_2CH$_3$), 4.18 (q, 2H, J = 7.2 Hz, ArOCH_2CH$_3$), 7.03 (dd, 1H, J = 8.7 Hz, J = 2.4 Hz, CH Ar), 7.22 (d, 1H, J = 2.4 Hz, CH Ar), 7.83 (d, 1H, J = 8.7 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3CH$_2$O), 15.0 (CH_3CH$_2$OAr), 26.5 (C(CH_3)$_2$), 54.1 (C(CH$_3$)$_2$), 62.0 (OCH_2CH$_3$), 64.3 (ArOCH_2CH$_3$), 104.3, 116.2, and 123.6 (CH Ar), 138.6, 148.1, 157.3, and 158.0 (C Ar), 173.5 (C=O). Anal. ($C_{15}H_{19}NO_3S_2$) C, H, N.

Ethyl 2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]propanoate [(*rac*)-11l]. Yellowish oil, 76% yield. IR (KBr) 1735 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.72 (d, 3H, J = 7.5 Hz, CH_3CH), 4.20 (q, 2H, J = 7.2 Hz, CH$_2$CH$_3$), 4.69 (q, 1H, J = 7.5 Hz, CH$_3$CH), 7.27−7.30 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz, CH Ar), 7.67 (d, 1H, J = 8.4 Hz, CH Ar), 7.84 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.3 (CH_3CH$_2$), 18.1 (CH_3CH), 45.4 (CH$_3$CH), 62.1 (CH$_2$CH$_3$), 121.8, 121.9, and 125.1 (CH Ar), 132.4 (CCl), 133.9 and 154.0 (C Ar), 166.9 (=C−S), 171.7 (C=O). Anal. ($C_{12}H_{12}ClNO_2S_2$) C, H, N.

Ethyl 2-[(5-Bromo-1,3-benzothiazol-2-yl)thio]propanoate [(*rac*)-11m]. Yellowish oil, 67% yield. IR (KBr) 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, J = 6.9 Hz, CH_3CH$_2$), 1.71 (d, 3H, J = 7.2 Hz, CH_3CH), 4.22 (q, 2H, J = 6.9 Hz, CH$_2$CH$_3$), 4.68 (q, 1H, J = 7.2 Hz, CH$_3$CH), 7.39−7.44 (dd, 1H, J = 8.7 Hz, J = 1.8 Hz, CH Ar), 7.60 (d, 1H, J = 8.7 Hz, CH Ar), 8.00 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.3 (CH_3CH$_2$), 18.1 (CH_3CH), 45.4 (CH$_3$CH), 62.1 (CH$_2$CH$_3$), 119.9 (CBr), 122.2, 124.8, and 127.7 (CH Ar), 134.5 and 145.1 (C Ar), 154.3 (=C−S), 171.6 (C=O). Anal. ($C_{12}H_{12}BrNO_2S_2$) C, H, N.

Ethyl 2-[(5-Cyano-1,3-benzothiazol-2-yl)thio]propanoate [(*rac*)-11n]. Yellowish oil, 78% yield. IR (KBr) 2234, 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, J = 6.9 Hz, CH_3CH$_2$), 1.72 (d, 3H, J = 7.2 Hz, CH_3CH), 4.25 (q, 2H, J = 6.9 Hz, CH$_2$CH$_3$), 4.73 (q, 1H, J = 7.2 Hz, CH$_3$CH), 7.52−7.56 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz, CH Ar), 7.85 (d, 1H, J = 8.4 Hz, CH Ar), 8,11 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.3 (CH_3CH$_2$), 18.1 (CH_3CH), 45.5 (CH$_3$CH), 62.2 (CH$_2$CH$_3$), 110.0 (CCN), 118.9 (CN), 122.3, 125.6, and 127.1 (CH Ar), 140.7 and 152.8 (C Ar), 168.3 (=C−S), 171.4 (C=O). Anal. ($C_{13}H_{12}N_2O_2S_2$) C, H, N.

Ethyl 2-[(5-Methoxy-1,3-benzothiazol-2-yl)thio]propanoate [(*rac*)-11o]. White oil, 60% yield. IR (KBr) 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, J = 6.9 Hz, CH_3CH$_2$), 1.70 (d, 3H, J = 7.2 Hz, CH_3CH), 3.86 (s, 3H, OCH_3), 4.21 (q, 2H, J = 6.9 Hz, CH$_2$CH$_3$), 4.64 (q, 1H, J = 7.2 Hz, CH$_3$CH), 6.96−6.97 (dd, 1H, J = 8.7 Hz, J = 2.4 Hz, CH Ar), 7.38 (d, 1H, J = 2.4 Hz, CH Ar), 7.60 (d, 1H, J = 8.7 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.3 (CH_3CH$_2$), 18.2 (CH_3CH), 45.4 (CH$_3$CH), 55.8 (OCH_3), 62.0 (CH$_2$CH$_3$), 104.9, 114.6, and 121.4 (CH Ar), 127.4, 154.4, and 159.1 (C Ar), 165.5 (=C−S), 171.9 (C=O). Anal. ($C_{13}H_{15}NO_3S_2$) C, H, N.

Ethyl 2-[(6-Bromo-1,3-benzothiazol-2-yl)thio]propanoate [(*rac*)-11p]. Yellowish oil, 52% yield. IR (KBr) 1741 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.70 (d, 3H, CH_3CH), 4.22 (q, 2H, J = 7.2 Hz, CH$_2$CH$_3$), 4.66 (q, 1H, J = 7.2 Hz, CH$_3$CH), 7.51 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz, CH Ar), 7.70 (d, 1H, J = 8.4 Hz, CH Ar), 7.88 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.3 (CH_3CH$_2$), 18.1 (CH_3CH), 45.4 (CH), 62.1 (CH_2CH$_3$), 118.1 (C Ar), 123.0, 123.8, and 129.7 (CH Ar), 137.3, 152.1, and 165.4 (C Ar), 171.6 (C=O). Anal. ($C_{12}H_{12}BrNO_2S_2$) C, H, N.

Ethyl 2-[(6-Ethoxy-1,3-benzothiazol-2-yl)thio]propanoate [(*rac*)-11q]. Yellowish oil, 47% yield. IR (KBr) 1734 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7.2 Hz, CH_3CH$_2$O), 1.43 (t, 3H, J = 7.2 Hz, CH_3CH$_2$OAr), 1.68 (d, 3H, CH_3CH), 4.05 (q, 2H, J = 7.2 Hz, OCH_2CH$_3$), 4.19 (m, 2H, ArOCH_2CH$_3$), 4.56 (q, 2H, J = 7.2 Hz, CHCH$_3$), 7.00 (dd, 1H, J = 8.7 Hz, J = 2.4 Hz, CH Ar), 7.21 (d, 1H, J = 2.4 Hz, CH Ar), 7.75 (d, 1H, J = 8.7 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.3 (CH_3CH$_2$O), 15.0 (CH_3CH$_2$OAr), 18.1 (CH_3CH), 45.5 (CHCH$_3$), 62.0 (OCH_2CH$_3$), 64.3 (ArOCH_2CH$_3$), 104.8, 115.7, and 122.6 (CH Ar), 137.3, 147.8, 156.8, and 160.7 (C Ar), 171.9 (C=O). Anal. ($C_{14}H_{17}NO_3S_2$) C, H, N.

Ethyl 2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]pentanoate [(*rac*)-11r]. Colorless oil, 63% yield. IR (KBr) 1731 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.26 (t, 3H, J = 6.9 Hz, CH_3CH$_2$O), 1.45−1.53 (m, 2H, CH$_2$CH$_3$), 1.88−2.10 (m, 2H, CH_2CH), 4.23 (dq, 2H, J = 7.2 Hz, OCH_2CH$_3$), 4.62 (t, 1H, J = 7.2 Hz, CHCH$_2$), 7.27 (dd, 1H, J = 8.7 Hz, J = 2.1 Hz, CH Ar), 7.65 (d, 1H, J = 8.7 Hz, CH Ar), 7.83 (d, 1H, J = 2.1 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 13.8 (CH_3CH$_2$), 14.3 (CH_3CH$_2$O), 20.5 (CH_2CH$_3$), 34.2 (CH_2CH), 50.3 (CH), 61.9 (OCH_2), 121.8 and 125.0 (CH Ar), 132.3, 133.9, 154.0, and 167.2 (C Ar), 171.4 (C=O). Anal. ($C_{14}H_{16}ClNO_2S_2$) C, H, N.

Ethyl 2-[(6-Ethoxy-1,3-benzothiazol-2-yl)thio]pentanoate [(*rac*)-11s]. Yellowish oil, 54% yield. IR (KBr) 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H, J = 7.2 Hz, CH_3CH$_2$CH$_2$), 1.24 (t, 3H, J = 6.9 Hz, CH_3CH$_2$O), 1.43 (t, 3H, J = 6.9 Hz, CH_3CH$_2$OAr), 1.46−1.57 (m, 2H, CH$_3$CH_2CH$_2$), 1.86−1.98 (m, 2H, CH$_3$CH$_2$-CH_2), 4.05 (q, 2H, J = 6.9 Hz, OCH_2CH$_3$), 4.20 (dq, 2H, ArOCH_2CH$_3$), 4.49 (t, 1H, J = 7.2 Hz, CHCH$_2$), 7.00 (dd, 1H, J = 9.0 Hz, J = 2.4 Hz, CH Ar), 7.21 (d, 1H, J = 2.4 Hz, CH Ar), 7.74 (d, 1H, J = 9.0 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 13.8 (CH_3CH$_2$CH$_2$), 14.3 (CH_3CH$_2$O), 15.0 (CH_3CH$_2$OAr), 20.6 (CH$_2$CH_2CH$_3$), 34.3 (CH$_2$CH$_2$CH$_3$), 50.5 (CHCH$_2$), 61.8 (OCH_2CH$_3$), 64.3 (ArOCH_2CH$_3$), 104.8, 115.7, and 122.6 (CH Ar), 137.3, 147.7, 156.8, and 161.0 (C Ar), 171.7 (C=O). Anal. ($C_{16}H_{21}NO_3S_2$) C, H, N.

Ethyl 2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]octanoate [(*rac*)-11t]. Yellowish oil, 55% yield. IR (KBr) 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.26 (t, 3H, J = 7.2 Hz, CH_3CH$_2$O), 1.28−1.50 (m, 8H, CH_2), 1.94−2.06 (m, 2H, CH_2), 4.22 and 4.23 (dq, 2H, J = 7.2 Hz, OCH_2CH$_3$), 4.60 (t, 1H, J = 7.2 Hz, CHS), 7.27 (dd, 1H, J = 8.7 Hz, J = 2.1 Hz, CH Ar), 7.65 (d, 1H, J = 8.7 Hz, CH Ar), 7.83 (d, 1H, J = 2.1 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3CH$_2$), 14.4 (CH_3CH$_2$O), 22.7, 27.1, 28.9, 31.7, and 32.2 (CH_2), 50.5 (CH), 62.0 (OCH_2), 121.8, 121.9, and 125.0 (CH Ar), 132.3, 133.9, 154.0, and 167.2 (C Ar), 171.4 (C=O). Anal. ($C_{17}H_{22}ClNO_2S_2$) C, H, N.

Ethyl [(5-Chloro-1,3-benzothiazol-2-yl)thio](phenyl)acetate [(*rac*)-11u]. Colorless oil, 64% yield. IR (KBr) 1738 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, J = 6.9 Hz, CH_3CH$_2$), 4.19 and 4.28 (dq, 2H, J = 6.9 Hz, OCH_2CH$_3$), 5.76 (s, 1H, CHS), 7.25−7.38 (m, 5H, CH Ar), 7.52 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz, CH Ar), 7.64 (d, 1H, J = 8.4 Hz, CH Ar), 7.83 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.3 (CH_3CH$_2$), 54.8 (CHS), 62.5 (OCH_2), 121.7, 121.9, 125.0, 128.7, 129.1, and 129.2 (CH Ar), 132.3, 133.9, 153.9, and 167.0 (C Ar), 169.6 (C=O). Anal. ($C_{17}H_{14}ClNO_2S_2$) C, H, N.

General Procedure for the Preparation of Acids 12c−k and *rac*-12l−u. NaOH (1 N, 3.9 mmol) was added to esters **11c−k** and *rac*-**11l-u** (3.0 mmol) in EtOH (20 mL), and the mixture was stirred at room temperature for 10−15 h. The solvent was removed under reduced pressure, and the residue was poured into water (20 mL) and acidified with concentrated HCl at 0 °C.

The aqueous layer was extracted with dichloromethane (3 × 20 mL), and the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by crystallization or by column chromatography on silica gel (eluent cyclohexane/ethyl acetate, 1:1, or dichloromethane/methanol, 9:1), affording desired acids with good yields.

2-[(5-Bromo-1,3-benzothiazol-2-yl)thio]-2-methylpropanoic Acid (12c). White solid, 46% yield, mp 121−123 °C. IR (KBr) 1710 cm^{-1}; ^1H NMR (DMSO) δ 1.71 (s, 6H, C(CH_3)$_2$), 7.48−7.51 (dd, 1H, J = 8.7 Hz, J = 2.7 Hz, CH Ar), 7.91 (d, 1H, J = 2.7 Hz, CH Ar), 8.04 (d, 1H, J = 8.7 Hz, CH Ar); ^{13}C NMR (DMSO) δ 27.7 (C(CH$_3$)$_2$), 60.8 (C(CH$_3$)$_2$), 119.5 (CBr), 123.7, 124.3, and 127.7 (CH Ar), 134.8 and 155.0 (C Ar), 169.9 (=C−S), 176.1 (C=O). Anal. ($C_{11}H_{10}BrNO_2S_2$) C, H, N.

2-[(5-Cyano-1,3-benzothiazol-2-yl)thio]-2-methylpropanoic Acid (12d). White solid, 61% yield, mp 265 °C (dec). IR (KBr) 2230, 1712 cm^{-1}; ^1H NMR (DMSO) δ 1.74 (s, 6H, C(CH_3)$_2$), 7.72−7.76 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz, CH Ar), 8.20 (d, 1H, J = 8.4 Hz, CH Ar), 8.34 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR (DMSO) δ 27.6 (C(CH$_3$)$_2$), 60.1 (C(CH$_3$)$_2$), 109.5 (CCN), 123.8, 125.8, and 127.7 (CH Ar), 140.8 and 153.1 (C Ar), 170.6 (=C−S), 175.5 (C=O). Anal. ($C_{12}H_{10}N_2O_2S_2$) C, H, N.

2-[(5-Trifluoromethyl-1,3-benzothiazol-2-yl)thio]-2-methylpropanoic Acid (12e). White needles, 48% yield, mp 147−149 °C. IR (KBr) 1709 cm^{-1}; ^1H NMR (CD$_3$OD) δ 1.78 (s, 6H, C(CH_3)$_2$), 7.61 (dd, 1H, J = 9.0 Hz, J = 1.8 Hz, CH Ar), 8.06 (d, 1H, J = 9.0 Hz, CH Ar), 8.14 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR (CD$_3$OD) δ 25.8 (C(CH$_3$)$_2$), 55.1 (C(CH$_3$)$_2$), 118.4, 120.9, and 122.2 (CH Ar), 128.4 (CF$_3$), 139.7, 144.6, 152.9, and 167.2 (C Ar), 175.9 (C=O). Anal. ($C_{12}H_{10}F_3NO_2S_2$) C, H, N.

2-[(5-Methoxy-1,3-benzothiazol-2-yl)thio]-2-methylpropanoic Acid (12f). Yellowish solid (from cyclohexane), 58% yield, mp 131−133 °C. IR (KBr) 1710 cm^{-1}; ^1H NMR (CD$_3$OD) δ 1.71 (s, 6H, C(CH_3)$_2$), 3.86 (s, 3H, OCH_3), 7.01−7.05 (dd, 1H, J = 8.7 Hz, J = 2.7 Hz, CH Ar), 7.44 (d, 1H, J = 2.7 Hz, CH Ar), 7.73 (d, 1H, J = 8.7 Hz, CH Ar); ^{13}C NMR (CD$_3$OD) δ 25.6 (C(CH$_3$)$_2$), 54.1 (C(CH$_3$)$_2$), 54.8 (OCH$_3$), 104.5, 115.3, and 121.4 (CH Ar), 128.3, 154.5, and 159.5 (C Ar), 163.6 (=C−S), 175.2 (C=O). Anal. ($C_{12}H_{13}NO_3S_2$) C, H, N.

2-[(6-Chloro-1,3-benzothiazol-2-yl)thio]-2-methylpropanoic Acid (12g). White solid, 45% yield, mp 140−141 °C. IR (KBr) 1714 cm^{-1}; ^1H NMR (CDCl$_3$) δ 1.75 (s, 6H, C(CH_3)$_2$), 7.42 (dd, 1H, J = 9.0 Hz, J = 1.8 Hz, CH Ar), 7.76 (d, 1H, J = 1.8 Hz, CH Ar), 7.81 (d, 1H, J = 9.0 Hz, CH Ar); ^{13}C NMR (CDCl$_3$) δ 26.0 (C(CH$_3$)$_2$), 54.6 (C(CH$_3$)$_2$), 121.1, 122.4, and 127.8 (CH Ar), 131.9, 136.2, 150.1, and 166.4 (C Ar), 174.3 (C=O). Anal. ($C_{11}H_{10}ClNO_2S_2$) C, H, N.

2-[(6-Bromo-1,3-benzothiazol-2-yl)thio]-2-methylpropanoic Acid (12h). White solid, 51% yield, mp 118−120 °C. IR (KBr) 1716 cm^{-1}; ^1H NMR (CDCl$_3$) δ 1.71 (s, 6H, C(CH_3)$_2$), 7.49 (dd, 1H, J = 8.7 Hz, J = 1.8 Hz, CH Ar), 7.71 (d, 1H, J = 8.7 Hz, CH Ar), 7.85 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR (CDCl$_3$) δ 26.2 (C(CH$_3$)$_2$), 55.2 (C(CH$_3$)$_2$), 119.3 (C Ar),122.9, 123.8, and 130.3 (CH Ar), 136.9, 150.8, and 165.9 (C Ar), 174.5 (C=O). Anal. ($C_{11}H_{10}BrNO_2S_2$) C, H, N.

2-[(6-Cyano-1,3-benzothiazol-2-yl)thio]-2-methylpropanoic acid (12i). White solid, 37% yield, mp 170−172 °C. IR (KBr) 2230, 1704 cm^{-1}; ^1H NMR (CD$_3$OD) δ 1.79 (s, 6H, C(CH_3)$_2$), 7.73 (dd, 1H, J = 8.4 Hz, J = 1.5 Hz, CH Ar), 7.94 (d, 1H, J = 8.4 Hz, CH Ar), 8.31 (d, 1H, J = 1.5 Hz, CH Ar); ^{13}C NMR (CD$_3$OD) δ 25.7 (C(CH$_3$)$_2$), 55.3 (C(CH$_3$)$_2$), 107.8 (C Ar), 118.4 (CN), 122.2, 126.1, and 129.5 (CH Ar), 136.3, 155.6, and 170.1 (C Ar), 175.5 (C=O). Anal. ($C_{12}H_{10}N_2O_2S_2$) C, H, N.

2-[(6-Trifluoromethyl-1,3-benzothiazol-2-yl)thio]-2-methylpropanoic Acid (12j). White needles, 42% yield, mp 149−151 °C. IR (KBr) 1709 cm^{-1}; ^1H NMR (CD$_3$OD) δ 1.78 (s, 6H, C(CH_3)$_2$), 7.68 (dd, 1H, J = 9.0 Hz, J = 1.8 Hz, CH Ar), 7.96 (d, 1H, J = 9.0 Hz, CH Ar), 8.21 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR (CD$_3$OD) δ 26.2 (C(CH$_3$)$_2$), 57.1 (C(CH$_3$)$_2$), 118.8, 121.9, and 122.9 (CH Ar), 126.3 (CF$_3$), 129.3, 136.1, 155.4, and 169.6 (C Ar), 177.9 (C=O). Anal. ($C_{12}H_{10}F_3NO_2S_2$) C, H, N.

2-[(6-Ethoxy-1,3-benzothiazol-2-yl)thio]-2-methylpropanoic Acid (12k). Yellowish solid, 46% yield, mp 124−126 °C. IR (KBr) 1716 cm^{-1}; ^1H NMR (CD$_3$OD) δ 1.40 (t, 3H, J = 7.2 Hz, CH_3CH$_2$OAr), 1.66 (s, 6H, C(CH_3)$_2$), 4.06 (q, 2H, J = 7.2 Hz, ArOCH_2CH$_3$), 7.06 (dd, 1H, J = 9.3 Hz, J = 2.4 Hz, CH Ar), 7.39 (d, 1H, J = 2.4 Hz, CH Ar), 7.78 (d, 1H, J = 9.3 Hz, CH Ar); ^{13}C NMR (CD$_3$OD) δ 13.9 (CH$_3$CH$_2$OAr), 25.8 (C(CH$_3$)$_2$), 54.5 (C(CH$_3$)$_2$), 64.0 (ArOCH$_2$CH$_3$), 104.1, 116.3, and 122.7 (CH Ar), 138.6, 147.5, 157.7, and 159.1 (C Ar), 175.9 (C=O). Anal. ($C_{13}H_{15}NO_3S_2$) C, H, N.

2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]propanoic Acid [*rac*-(12l)]. White solid, 46% yield, mp 110−112 °C. IR (KBr) 1709 cm^{-1}; ^1H NMR (CD$_3$OD) δ 0.16 (d, 3H, J = 6.9 Hz, CH_3CH), 3.02 (q, 1H, J = 6.9 Hz, CH$_3$CH), 5.73−5.76 (dd, 1H, J = 8.7 Hz, J = 1.8 CH Ar), 6.22 (d, 1H, J = 1.8 Hz, CH Ar), 6.25 (d, 1H, J = 8.7 Hz, CH Ar); ^{13}C NMR (CD$_3$OD) δ 20.8 (CH$_3$CH), 51.1 (CH$_3$$C$H), 121.0, 123.6, and 124.8 (CH Ar), 131.6 and 154.5 (C Ar), 133.9 (CCl), 171.4 (=C−S), 173.4 (C=O). Anal. ($C_{10}H_8ClNO_2S_2$) C, H, N.

2-[(5-Bromo-1,3-benzothiazol-2-yl)thio]propanoic Acid [*rac*-(12m)]. Yellow solid, 78% yield, mp 108−109 °C. IR (KBr) 1712 cm^{-1}; ^1H NMR (DMSO) δ 1.62 (d, 3H, J = 6.6 Hz, CH_3CH), 4.37 (q, 1H, J = 6.6 Hz, CH$_3$CH), 7.45−7.49 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz, CH Ar), 7.92 (d, 1H, J = 8.4 Hz, CH Ar), 7.99 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR (DMSO) δ 20.6 (CH$_3$CH), 50.6 (CH$_3$$C$H), 119.7 ($C$Br), 123.9, 124.0, and 127.5 (CH Ar), 134.4 and 154.8(C Ar), 170.8 (=C−S), 173.5 (C=O). Anal. ($C_{10}H_8BrNO_2S_2$) C, H, N.

2-[(5-Cyano-1,3-benzothiazol-2-yl)thio]propanoic Acid [*rac*-(12n)]. White solid, 66% yield, mp 124−125 °C. IR (KBr) 2233, 1721 cm^{-1}; ^1H NMR (DMSO) δ 1.63 (d, 3H, J = 6.9 Hz, CH_3CH), 4.45 (q, 1H, J = 6.9 Hz, CH$_3$CH), 7.70−7.73 (dd, 1H, J = 8.1 Hz, J = 1.5 Hz, CH Ar), 8.19 (d, 1H, J = 8.1 Hz, CH Ar), 8.29 (d, 1H, J = 1.5 Hz, CH Ar); ^{13}C NMR (DMSO) δ 20.1 (CH$_3$CH), 49.6 (CH$_3$$C$H), 109.5 ($C$CN), 120.7 ($C$N), 123.9, 125.3, and 127.5 (CH Ar), 140.6 and 153.1 (C Ar), 171.2 (=C−S), 173.3 (C=O). Anal. ($C_{11}H_8N_2O_2S_2$) C, H, N.

2-[(5-Methoxy1,3-benzothiazol-2-yl)thio]propanoic Acid [*rac*-(12o)]. White solid, 90% yield, mp 131−133 °C. IR (KBr) 1710 cm^{-1}; ^1H NMR (CD$_3$OD) δ 1.70 (d, 3H, J = 7.5 Hz, CH_3CH), 3.84 (s, 3H, OCH_3), 4.48 (q, 1H, J = 7.5 Hz, CH$_3$CH), 6.93−6.97 (dd, 1H, J = 8.7 Hz, J = 2.4 Hz, CH Ar), 7.33 (d, 1H, J = 2.4 Hz, CH Ar), 7.67 (d, 1H, J = 8.7 Hz, CH Ar); ^{13}C NMR (CD$_3$OD) δ 18.9 (CH$_3$CH), 49.3 (CH$_3$$C$H), 54.8 (OCH$_3$), 104.0, 113.8, and 121.2 (CH Ar), 126.6, 154.4, and 159.4 (C Ar), 155.8 (=C−S), 168.9 (C=O). Anal. ($C_{11}H_{11}NO_3S_2$) C, H, N.

2-[(6-Bromo-1,3-benzothiazol-2-yl)thio]propanoic Acid [*rac*-(12p)]. White solid, 63% yield, mp 112−114 °C. IR (KBr) 1718 cm^{-1}; ^1H NMR (DMSO) δ 1.61 (d, 3H, J = 7.2 Hz, CH_3CH), 4.45 (q, 1H, J = 7.2 Hz, CHCH$_3$), 7.55 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz, CH Ar), 7.72 (d, 1H, J = 8.4 Hz, CH Ar), 8.24 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR (DMSO) δ 20.0 (CH$_3$CH), 49.1 (CH), 117.4 (C Ar), 123.1, 124.9, and 130.0 (CH Ar), 137.2, 152.5, and 168.6 (C Ar), 173.3 (C=O). Anal. ($C_{10}H_8BrNO_2S_2$) C, H, N.

2-[(6-Ethoxy-1,3-benzothiazol-2-yl)thio]propanoic Acid [*rac*-(12q)]. White solid, 49% yield, mp 90−91 °C. IR (KBr) 1716 cm^{-1}; ^1H NMR (CDCl$_3$) δ 1.45 (t, 3H, J = 6.9 Hz, CH_3CH$_2$OAr), 1.64 (d, 3H, CH_3CH), 4.07 (q, 2H, J = 6.9 Hz, ArOCH_2CH$_3$), 4.32 (q, 2H, J = 6.9 Hz, CHCH$_3$), 7.06 (dd, 1H, J = 9.0 Hz, J = 2.4 Hz, CH Ar), 7.23 (d, 1H, J = 2.4 Hz, CH Ar), 7.75 (d, 1H, J = 9.3 Hz, CH Ar); ^{13}C NMR (CDCl$_3$) δ 14.9 (CH$_3$CH$_2$OAr), 16.4 (CH$_3$CH), 44.7 (CHCH$_3$), 64.4 (ArOCH$_2$CH$_3$), 105.0, 116.5, and 121.7 (CH Ar), 136.2, 145.3, 157.5, and 160.5 (C Ar), 171.9 (C=O). Anal. ($C_{12}H_{13}NO_3S_2$) C, H, N.

2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]pentanoic Acid [*rac*-(12r)]. White solid, 59% yield, mp 100−101 °C. IR (KBr) 1718 cm^{-1}; ^1H NMR (CDCl$_3$) δ 0.97 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.51−1.61 (m, 2H, CH_2CH$_3$), 1.83−2.19 (m, 2H, CH_2CH), 4.35 (t, 1H, J = 7.2 Hz, CH), 7.34 (dd, 1H, J = 8.7 Hz, J = 2.1 Hz, CH Ar), 7.69 (d, 1H, J = 8.7 Hz, CH Ar), 7.87 (d, 1H, J = 2.1 Hz, CH Ar); ^{13}C NMR (CDCl$_3$) δ 13.8 (CH$_3$CH$_2$), 20.6 (CH$_2$CH$_3$), 34.6 (CH$_2$CH), 49.8 (CH), 121.4, 122.1, and 125.9

(*C*H Ar), 133.1, 133.3, 152.3, and 169.2 (*C* Ar), 172.5 (*C*=O). Anal. ($C_{12}H_{12}ClNO_2S_2$) C, H, N.

2-[(6-Ethoxy-1,3-benzothiazol-2-yl)thio]pentanoic Acid [*rac*-(12s)]. Yellowish oil, 51% yield. IR (KBr) 1718 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (t, 3H, J = 7.2 Hz, CH_3CH$_2$CH$_2$), 1.44 (t, 3H, J = 6.9 Hz, CH_3CH$_2$OAr), 1.42−1.57 (m, 2H, CH$_3$CH_2CH$_2$), 1.78−2.20 (m, 2H, CH$_3$CH$_2$CH_2), 4.06 (q, 2H, J = 6.9 Hz, ArOCH_2CH$_3$), 4.17 (t, 1H, J = 7.2 Hz, CHCH$_2$), 7.04 (dd, 1H, J = 9.0 Hz, J = 2.4 Hz, CH Ar), 7.21 (d, 1H, J = 2.4 Hz, CH Ar), 7.75 (d, 1H, J = 9.0 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 13.8 (*C*H$_3$CH$_2$CH$_2$), 14.9 (*C*H$_3$CH$_2$OAr), 20.6 (CH$_2$*C*H$_2$CH$_3$), 32.4 (*C*H$_2$CH$_2$CH$_3$), 50.1 (*C*HCH$_2$), 64.4 (ArO*C*H$_2$CH$_3$), 105.0, 116.4, and 121.8 (*C*H Ar), 136.4, 145.6, 157.4, and 164.8 (*C* Ar), 172.2 (*C*=O). Anal. ($C_{14}H_{17}NO_3S_2$) C, H, N.

2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]octanoic Acid [*rac*-(12t)]. White solid, 53% yield, mp 91−93 °C. IR (KBr) 1716 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.24−1.29 (m, 6H, CH_2), 1.42−1.59 (m, 2H, CH_2), 1.92−2.05 (m, 2H, CH_2), 4.28 (t, 1H, J = 7.2 Hz, CHS), 7.36 (dd, 1H, J = 8.7 Hz, J = 2.1 Hz, CH Ar), 7.70 (d, 1H, J = 8.7 Hz, CH Ar), 7.88 (d, 1H, J = 2.1 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.2 (*C*H$_3$CH$_2$), 22.7, 27.3, 29.0, 30.5, and 31.6 (*C*H$_2$), 50.2 (*C*H), 121.3, 122.2, and 126.0 (*C*H Ar), 133.2, 133.3, 152.1, and 170.4 (*C* Ar), 171.7 (*C*=O). Anal. ($C_{15}H_{18}ClNO_2S_2$) C, H, N.

[(5-Chloro-1,3-benzothiazol-2-yl)thio](phenyl)acetic Acid [*rac*-(12u)]. White needles (from cyclohexane), 56% yield, mp 186−188 °C. IR (KBr) 1707 cm^{-1}; ^1H NMR (CD_3OD) δ 5.79 (s, 1H, CHS), 7.31−7.41 (m, 5H, CH Ar), 7.54 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz, CH Ar), 7.81 (d, 1H, J = 8.4 Hz, CH Ar), 7.84 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR (CD_3OD) δ 55.0 (*C*HS), 120.9, 122.2, 124.8, 128.4, 128.7, and 128.9 (*C*H Ar), 132.2, 133.8, 135.0, 153.8, and 167.9 (*C* Ar), 171.2 (*C*=O). Anal. ($C_{15}H_{10}ClNO_2S_2$) C, H, N.

General Procedure for the Preparation of (*R*)- and (*S*)-Ethyl 2-Bromopropanoate. Absolute EtOH (37.16 mmol), toluene (31.63 mmol), and concentrated H_2SO_4 (0.265 mmol) were added to (*R*)- or (*S*)-2-bromopropanoic acid (22.12 mmol, 3.39 g) using a Dean−Stark apparatus. After the mixture was stirred for 5 h at reflux, absolute EtOH (6.64 mmol) was added and the reaction mixture was stirred for another 30 min, then poured into water (10 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with $NaHCO_3$ (50 mL) and H_2O (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude products were used without purification.

General Procedure for the Preparation of Arylthioisobutanoic Esters (*R*)- and (*S*)-11l and (*R*)- and (*S*)-11o. The heterocyclic thiol **10l** or **10o** (2.21 mmol) and (*R*)- or (*S*)-ethyl 2-bromopropanoate (2.21 mmol) dissolved in absolute EtOH (10 mL) were added to a solution of sodium (50.8 mg, 2.21 mmol) in absolute EtOH (5 mL) under nitrogen atmosphere. After the mixture was stirred for 2 h at reflux, the solvent was removed under reduced pressure. The residue was poured into water (20 mL) and extracted with diethyl ether (3 × 20 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude products were used without purification.

(*R*)-Ethyl 2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]propanoate [(*R*)-(11l)]. Yellowish oil, 80% yield, $[\alpha]_D^{20}$ +118.7° (*c* 1.01, $CHCl_3$). IR (KBr) 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.72 (d, 3H, J = 7.5 Hz, CH_3CH), 4.20 (q, 2H, J = 7.2 Hz, CH_2CH$_3$), 4.69 (q, 1H, J = 7.5 Hz, CH$_3$CH), 7.27−7.30 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz, CH Ar), 7.67 (d, 1H, J = 8.4 Hz, CH Ar), 7.84 (d, 1H, J = 1.8 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.3 (*C*H$_3$CH$_2$), 18.1 (*C*H$_3$CH), 45.4 (CH$_3$*C*H), 62.1 (*C*H$_2$CH$_3$), 121.8, 121.9, and 125.1 (*C*H Ar), 132.4 (*C*Cl), 133.9 and 154.0 (*C* Ar), 166.9 (=*C*−S), 171.7 (*C*=O). Anal. ($C_{12}H_{12}ClNO_2S_2$) C, H, N.

(*S*)-Ethyl 2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]propanoate [(*S*)-(11l)]. Yellowish oil, 61% yield, $[\alpha]_D^{24}$ −107.6° (*c* 1.05, $CHCl_3$). Spectroscopic data are the same as for the (*R*)-enantiomer.

(*R*)-Ethyl 2-[(5-methoxy-1,3-benzothiazol-2-yl)thio]propanoate [(*R*)-(11o)]. White oil, 57% yield, $[\alpha]_D^{21}$ +98.7° (*c* 1.68, $CHCl_3$). IR (KBr) 1732 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (t, 3H, J = 6.9 Hz, CH_3CH$_2$), 1.70 (d, 3H, J = 7.2, CH_3CH), 3.86 (s, 3H, OCH_3), 4.21 (q, 2H, J = 6.9 Hz, CH_2CH$_3$), 4.64 (q, 1H, J = 7.2 Hz, CH$_3$CH), 6.96−6.97 (dd, 1H, J = 8.7 Hz, J = 2.4 Hz, CH Ar), 7.38 (d, 1H, J = 8.7 Hz, CH Ar), 7.60 (d, 1H, J = 2.4 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 14.3 (*C*H$_3$CH$_2$), 18.2 (*C*H$_3$CH), 45.4 (CH$_3$*C*H), 55.8 (O*C*H$_3$), 62.0 (*C*H$_2$CH$_3$), 104.9, 114.6, and 121.4 (*C*H Ar), 127.4, 154.4, and 159.1 (*C* Ar), 165.5 (=*C*−S), 171.9 (*C*=O). Anal. ($C_{13}H_{15}NO_3S_2$) C, H, N.

(*S*)-Ethyl 2-[(5-Methoxy-1,3-benzothiazol-2-yl)thio]propanoate [(*S*)-(11o)]. White oil, 60% yield, $[\alpha]_D^{21}$ −100.7° (*c* 1.68, $CHCl_3$). Spectroscopic data are the same as for the (*R*)-enantiomer.

General Procedure for the Preparation of Acids (*R*)- and (*S*)-12l and (*R*)- and (*S*)-12o. HCl (6 N, 23 mmol) was added to chiral esters (*R*)- and (*S*)-**11l** and (*R*)- and (*S*)-**11o** (1.22 mmol, 367.4 mg) at room temperature. Then the mixture was heated at 60 °C for 24 h. Water (10 mL) was added to the reaction mixture and then was extracted with diethyl ether (3 × 15 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent, 100% dichloromethane), affording acids with good yields.

(*R*)-2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]propanoic Acid [(*R*)-(12l)]. White solid, 87% yield, $[\alpha]_D^{19}$ +28.7 (*c* 0.75, MeOH), mp 108−110 °C. IR (KBr) 1709 cm^{-1}; ^1H NMR (CD_3OD) δ 0.16 (d, 3H, J = 6.9 Hz, CH_3CH), 3.02 (q, 1H, J = 6.9 Hz, CH$_3$CH), 5.73−5.76 (dd, 1H, J = 8.7 Hz, J= 1.8 Hz, CH Ar), 6.22 (d, 1H, J = 1.8 Hz, CH Ar), 6.25 (d, 1H, J = 8.7 Hz, CH Ar); ^{13}C NMR (CD_3OD) δ 20.8 (*C*H$_3$CH), 51.1 (CH$_3$*C*H), 121.0, 123.6, and 124.8 (*C*H Ar), 131.6 and 154.5 (*C* Ar), 133.9 (*C*Cl), 171.4 (=*C*−S), 173.4 (*C*=O). Anal. ($C_{10}H_8ClNO_2S_2$) C, H, N.

(*S*)-2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]propanoic Acid [(*S*)-(12l)]. White solid, 48% yield, $[\alpha]_D^{31}$ −34.9° (*c* 0.75, MeOH). Spectroscopic data are the same for the (*R*)-enantiomer.

(*R*)-2-[(5-Methoxy-1,3-benzothiazol-2-yl)thio]propanoic Acid [(*R*)-(12o)]. White solid, 93% yield, $[\alpha]_D^{24}$ +17.8° (*c* 1.05, MeOH), mp 134−135 °C. IR (KBr) 1710 cm^{-1}; ^1H NMR (CD_3OD) δ 1.70 (d, 3H, J = 7.5 Hz, CH_3CH), 3.84 (s, 3H, OCH_3), 4.48 (q, 1H, J = 7.5 Hz, CH$_3$CH), 6.93−6.97 (dd, 1H, J = 8.7 Hz, J = 2.4 Hz, CH Ar), 7.33 (d, 1H, J = 2.4 Hz, CH Ar), 7.67 (d, 1H, J = 8.7 Hz, CH Ar); ^{13}C NMR (CD_3OD) δ 18.9 (*C*H$_3$CH), 49.3 (CH$_3$*C*H), 54.8 (O*C*H$_3$), 104.0, 113.8, and 121.2 (*C*H Ar), 126.6, 154.4, and 159.4 (*C* Ar), 155.8 (=*C*−S), 168.9 (*C*=O). Anal. ($C_{11}H_{11}NO_3S_2$) C, H, N.

(*S*)-2-[(5-Methoxy-1,3-benzothiazol-2-yl)thio]propanoic Acid [(*S*)-(12o)]. White solid, 85% yield, $[\alpha]_D^{24}$ −17.3° (*c* 0.79, MeOH), mp 134−135 °C. IR (KBr) 1710 cm^{-1}; ^1H NMR (CD_3OD) δ 1.70 (d, 3H, J = 7.5 Hz, CH_3CH), 3.84 (s, 3H, OCH_3), 4.48 (q, 1H, J = 7.5 Hz, CH$_3$CH), 6.93−6.97 (dd, 1H, J = 8.7 Hz, J = 2.4 Hz, CH Ar), 7.33 (d, 1H, J = 2.4 Hz, CH Ar), 7.67 (d, 1H, J = 8.7 Hz, CH Ar); ^{13}C NMR (CD_3OD) δ 18.9 (*C*H$_3$CH), 49.3 (CH$_3$*C*H), 54.8 (O*C*H$_3$), 104.0, 113.8, and 121.2 (*C*H Ar), 126.6, 154.4, and 159.4 (*C* Ar), 155.8 (=*C*−S), 168.9 (*C*=O). Anal. ($C_{11}H_{11}NO_3S_2$) C, H, N.

Biology. Cell-Based Transactivation Assay. Human embryonic kidney 293 cell line (HEK293) was cultured in Dulbecco's modified Eagle's minimal essential medium containing 10% fetal calf serum and supplemented with penicillin/streptomycin, sodium pyruvate, and nonessential amino acids at 37 °C in a humidified atmosphere of 5% CO_2 in air. Ligand agonist activity for each PPAR subtype was determined by its transactivation activity in a cell-based reporter-gene assay. Transfections of PPAR and reporter gene constructs were performed by calcium phosphate coprecipitation. Before transfection, the culture medium was replaced by fresh serum-free medium, and 6 h after transfection, the ligand, dissolved in DMSO, was added. The final concentration of DMSO did not exceed 0.1% (v/v) in any of the samples. Eighteen hours after treatment, luciferase and renilla activities were measured by a dual luciferase assay kit

(Promega) using a luminometer (Labsystems Ascent Luminoskan reader). DNA cotransfection experiments included 50 ng of reporter plasmid, 20 ng of renilla, 40 ng of pGEM, and 30 ng of each receptor expression plasmid per well in a 96-well plate. Luciferase data were normalized to the internal renilla control, and reported values are the mean of triplicate assays. Luciferase activity was determined as fold activation relative to untreated cells.

RTqPCR. HepG2 was obtained from ATCC (ATCC-LGC Promochem, London, U.K.). Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS-GOLD and 1% penicillin/streptomycin at 37 °C in a humidified incubator with 5% CO_2 in air. For the treatment, 1×10^6 cells were seeded into six-well culture dishes, grown for 24 h, and finally treated with vehicle (DMSO) and ligand (150 μM). After incubation for 48 h, mRNA was extracted.

Total RNA was isolated by TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacture's instruction. To avoid possible DNA contaminations, RNA was treated with DNAase-1 (Ambion, Foster City, CA). RNA purity was checked by spectrophotometer and RNA integrity by examination on agarose gel electrophoresis. cDNA was synthesized retrotranscribing 4 μg of total RNA in a total volume of 100 μL using a high capacity DNA archive kit (Applied Biosystem, Foster City, CA) and following the manufacturer's instruction.

RTqPCR primers were designed using Primer Express software. RTqPCR assays were performed in 96-well optical reaction plates using the ABI 7500HT machine (Applied Biosystem). PCR assays were conducted in triplicate wells for each sample. Baseline values of amplification plots were set automatically, and threshold values were kept constant to obtain normalized cycle times and linear regression data. The following reaction mixture per well was used: 10 μL Power Syber Green (Applied Biosystem), 2.4 μL of primers at a final concentration of 150 nM, 4.6 μL of RNAase free water, 3 μL of cDNA (60 ng). For all experiments the following conditions were used: denaturation at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s, then at 60 °C for 60 s. Quantitative normalization of cDNA in each sample was performed using cyclophilin as internal control. Relative quantification was performed using the ΔΔCT method.

Validated primers for RTqPCR are listed below:

Human CPT1: FW-5′TGCCATGGATCTGCTGTATATCC3′, RV-5′GCGTTGCCGGCTCTTG3′

Human cyclophilin: FW-5′TTTCATCTGCACTGCCAAGA3′, RV-5′TTGCAAAACACCACATGCT3′

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