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Synthesis and SAR of selective benzothiophene, benzofuran, and indole-based peroxisome proliferator-activated receptor δ agonists

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Abstract—Recent literature has suggested the benefit of selective PPARδ agonists for the treatment of atherosclerosis and other disease states associated with the metabolic syndrome. Herein we report the synthesis and structure–activity relationships of a series of novel and selective PPARδ agonists. Our search began with identification of a novel benzothiophene template which was modified by the addition of various thiazolyl, isoxazolyl, and benzyloxy-benzyl moieties. Further elucidation of the SAR led to the identification of benzofuran and indole based templates. During the course of our research, we discovered three new chemical templates with varying degrees of affinity and potency for PPARδ versus the PPARα and PPARγ subtypes.

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor super family. The PPARs consist of three subtypes: PPARα, PPARγ, and PPARδ (also known as PPARβ). The PPARs' putative biological role is the maintenance of lipid and glucose homeostasis through downstream signaling to the biochemical machinery responsible for energy storage and catabolism.[1] PPARα, expressed primarily in the liver, is associated with lipoprotein catabolism. PPARγ, expressed primarily in adipose tissue, is a regulator of glucose and insulin homeostasis and fatty acid storage. The role of PPARδ, ubiquitously expressed, is less well understood but recent research has suggested a variety of biological functions centering around fatty acid oxidation in tissues with high energy demands such as heart, adipose, and skeletal muscle.[2a–d] PPARδ has begun to gain more attention as a molecular target for the treatment of cardiovascular diseases. Notable is a report which states that a selective PPARδ agonist increased HDLc by 80%, while reducing LDL-cholesterol by 29%, in rhesus monkeys.[3] The potential of PPARδ agonists as pharmaceutical agents for HDL-cholesterol elevation prompted our efforts to find novel and selective compounds.

Examination of the literature revealed few reports of selective PPARδ ligands. However, it was clear that the prototypical selective PPARδ agonists (Fig. 1), such as GW501516, L-165041, and L-783483, had either an acetoxy or acetic acid head group attached to an aryl core, with a variety of lipophilic tail portions.[4]

This was in contrast to the acidic head groups found in most PPARα and non-TZD PPARγ agonists. The α and γPPAR subtypes can accept and prefer bulky lipophilic substituents adjacent to the requisite carboxylic acid function. This is due to the relatively large hydrophobic pocket found adjacent to the activation domain in PPARα and PPARγ. The same hydrophobic pocket in PPARδ is considerably narrower and will not accommodate bulky substituents.[5] This indicated to us that a large component of PPARδ subtype specificity (but not exclusively) would be determined by the amount of steric crowding around the acidic head portion rather than what was found at the aryl core or lipophilic tail. Our strategy was to utilize a generic PPAR scaffold (Fig. 2) consisting of an acidic head group, aryl body, and lipophilic tail to construct new compounds.

Keywords: PPAR; Benzothiophene; Benzofuran; Indole.
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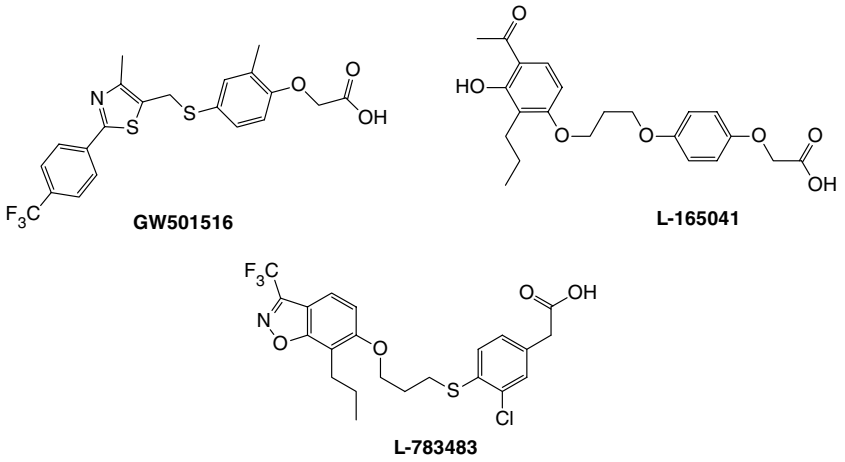


Figure 1. PPARδ selective agonists.

Specifically, in order to increase the binding affinity of our new ligands, we sought novel head/body combinations that would have at least one less degree of rotational freedom relative to the prototypical arylacetoxy and arylacetic acid motifs. The caveat to this approach being that the requisite carboxyl function still maintains its ability to fit in the relatively narrow activation domain of PPARδ.

A substructure search of our proprietary chemical library using these criteria initially led us to the 6-hydroxybenzothiophene-3-acetic acid core (**6**) and from there we derived the 6-hydroxybenzofuran (**8**), 5-hydroxy indole acetic acid (**13**), and 5-mercapto-indole-acetic acid (**15**) cores.

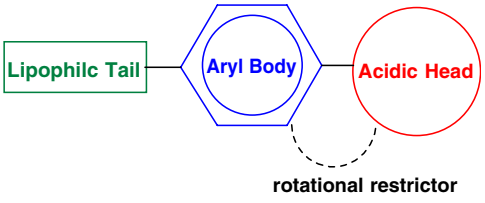
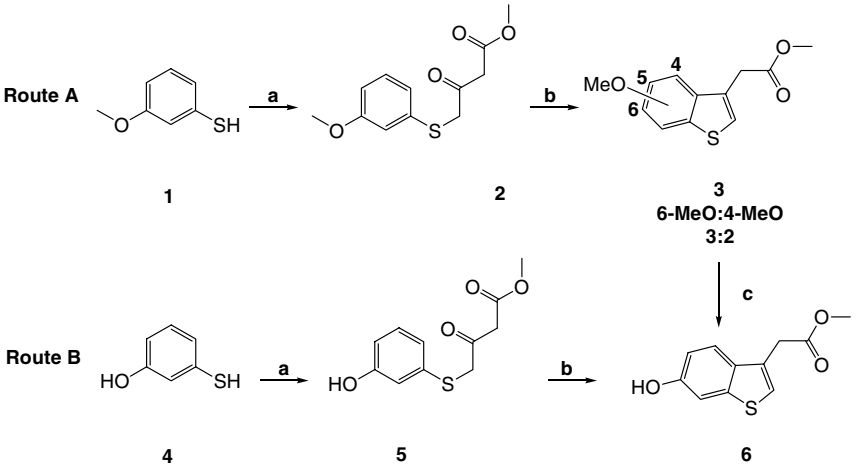


Figure 2. Prototypical PPAR ligand with rotational restrictor.

Once identified, we began to combine the new acidic head/aryl body combinations with a previously known tail and others discovered through screening of a combinatorial library.

The 6-hydroxybenzothiophene-3-acetic acid core was prepared by alkylation of 3-methoxythiophenol with methyl 4-chloroacetoacetate followed by an acid mediated cyclocondensation. The cyclization to the benzothiophene core resulted in a regioisomeric mixture of compounds in a 3:2 ratio (Scheme 1). The mixture of regioisomers could not be separated by recrystallization or chromatography, so it was used as is in the next step. De-methylation was accomplished using boron tribromide in dichloromethane at 0 °C. After work up, chromatography, and recrystallization, the desired 6-hydroxybenzothiophen-3-acetic acid core was isolated in 37% yield over 3 steps. Methyl and chloro substituted analogues of **6** were prepared in a similar manner. We



Scheme 1. Reagents and conditions: (a) methyl 4-chloroacetoacetate, Cs_2CO_3, CH_3CN/DMF 5:1; (b) methanesulfonic acid, DCM; (c) BBr_3, DCM, 0 °C. Route A, 37% over three steps; Route B, 34% over two steps.

later determined that it was not necessary to have the latent hydroxy group protected to arrive at **6**, and in fact it seemed preferable judging by the regiochemical ratio of the cyclocondensation step. In the event, selective alkylation of the thiol of 3-hydroxy thiophenol with methyl 4-chloroacetoacetate gave the intermediate acetoacetetate derivative **5**. Cyclization with excess methanesulfonic acid in dichloromethane at room temperature gave the desired 6-hydroxybenzothiophen-3-acetic acid core and only trace amounts of the regiochemical partner. After chromatography and recrystallization, **6** was isolated in 34% yield over two steps.

The benzofuran core was made by a two-step literature procedure (Scheme 2).[6]

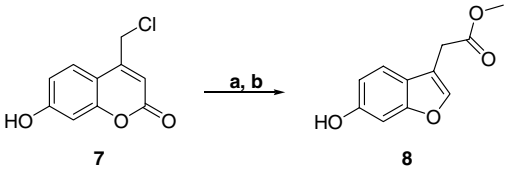
The lipophilic tails (Fig. 3) were either commercially available or were prepared according to literature procedures.[7]

The final compounds, **10a**–**t**, were then made in a simple two-step procedure starting with alkylation of the free phenol in acetonitrile with the chloride of the lipophilic tail using cesium carbonate as base (Scheme 3). After purification, the resulting esters **9a**–**t** were saponified with lithium hydroxide to give the desired carboxylic acids in good to excellent yields (Table 1).

The (5-mercapto-indol-1-yl)-acetic acid core was prepared by alkylating commercially available 5-benzyloxyindole in dimethylformamide with methyl bromoacetate using sodium hydride as base (Scheme 4). The resulting compound was then de-benzylated using hydrogen and palladium as catalyst. The thiol was introduced following the two-step Newman–Kwart procedure.[8] After trying several conditions to effect the rearrangement, we found that the best yields were obtained when the thiocarbamate in warm diphenyl ether was added to diphenyl ether at 259 °C.

The final indole compounds **16a**–**g** were then prepared in the same manner as described for compounds **10a**–**t** (Table 2).

The synthesized compounds **10a**–**t** and **16a**–**g** were evaluated for in vitro binding and agonist activity at hPPARδ using known methods.[9,10] We started our SAR by making analogues with the benzothiopheneacetic acid core.[11] The unsubstituted analogues (**10a** and **10b**) had a strong affinity for PPARδ versus PPARα. In general, we found that most of the compounds reported herein had very little affinity for PPARγ. The example of a benzothiopheneacetic acid core and lipophilic thiazole tail (**10a**) demonstrated potency and selectivity for PPARδ similar to that of GW501516. Replacing the lipophilic thiazole tail with a chlorophenyl isoxazole (**10b**) resulted in only a slight loss in potency but a greater degree of selectivity. The trifluoromethylphenyl isoxazole analogue (**10c**) had similar potency to the chlorophenyl analogue, but surprisingly had a greater affinity for PPARα. The benzofuran counterparts (**10d**–**f**) were less potent, perhaps due to the smaller size of oxygen relative to sulfur, leaving unfilled space within the PPARδ activation domain. By comparison the indole analogues **16a**–**b** were about 2- to 3-fold less potent and selective for PPARδ versus PPARα than the corresponding benzothiophene analogues. Substitution at the 4- and 5-position of the benzothiophene core in all cases resulted in a diminution of PPARδ potency. Compounds containing benzyloxybenzyl tails (**10n**–**t** and **16d**–**f**) represented an unprecedented addition to our stable of lipophilic tails. The



Scheme 2. Reagents and condition: (a) NaOH, reflux; (b) LiOH, $(MeO)_2SO_2$.

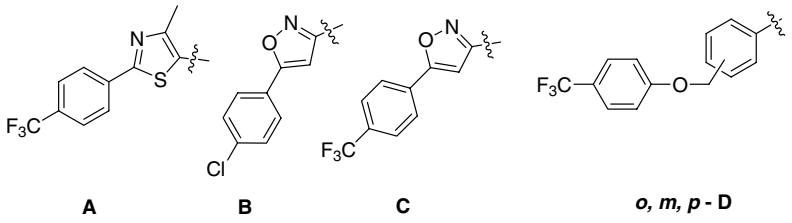
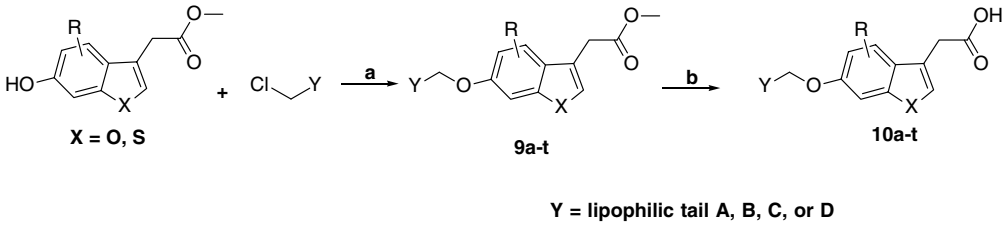
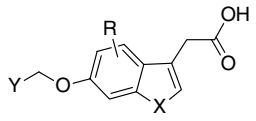


Figure 3. Lipophilic tails.



Scheme 3. Reagents and conditions: (a) Y-CH_2Cl, Cs_2CO_3, CH_3CN, 40–87%; (b) LiOH/water/THF, 55–96%.

Table 1. Activity of benzothiophene and benzofuran based compounds



10a-t

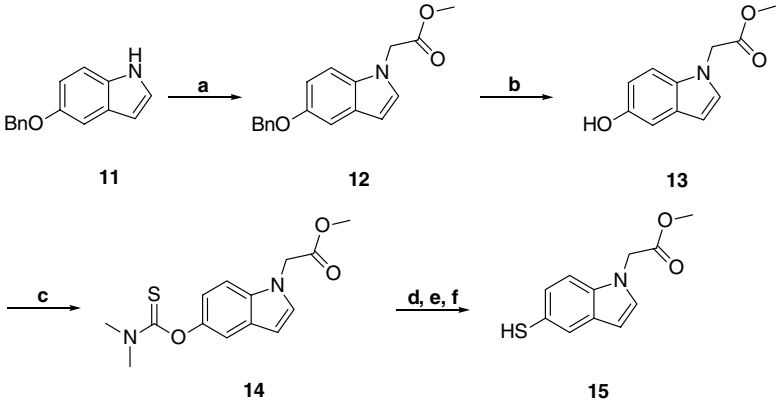
Compound	R	X	Y = lipophilic tail	IC_{50}[a,c] (nM)			Fold selectivity: hPPARδ vs. hPPARα	EC_{50}[b,c] hPPARδ (nM)
				hPPARδ	hPPARα	hPPARγ		
GW501506	—	—	—	2.6	463	NT	178	4.0
a	H	S	A	3.1	482	>10,000	155	14.7
b	H	S	B	7.7	3820	>10,000	496	226
c	H	S	C	11	261	NT	24	305
d	H	O	A	14	679	NT	49	243
e	H	O	B	81	2380	>10,000	29	323
f	H	O	C	25	1420	>10,000	57	1610
g	5-Me	S	A	237	683	6260	3	NT
h	4-Cl	S	A	58	1040	>10,000	18	227
i	4-Me	S	B	23	311	8660	14	638
j	5-Me	S	B	37	5810	NT	159	656
k	4-Cl	S	B	375	3840	NT	10	6360
l	4-Me	S	C	37	1470	>10,000	40	561
m	4-Cl	S	C	97	1350	NT	14	2630
n	H	S	*o*-D	244	7860	>10,000	32	2230
o	5-Me	S	*o*-D	43	4680	7580	108	172
p	H	S	*m*-D	15	963	5790	65	166
q	H	S	*p*-D	3.6	229	3730	64	1070
r	H	O	*o*-D	662	>10,000	>10,000	>15	3120
s	H	O	*m*-D	24.1	2500	5400	104	1490
t	H	O	*p*-D	81	2380	>10,000	29	3440

NT = not tested.

[a] Concentration that inhibits 50% of the interaction between the PPAR LBD and the radiolabeled ligand.

[b] Concentration of test compound which produced 50% of the maximal reporter activity.

[c] The results are based on at least two experiments, each dose done in triplicate (SD = 10%).

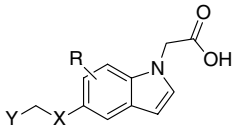


Scheme 4. Reagents and conditions: (a) methyl bromoacetate, NaH, CH_3CN/DMF; (b) H_2, 10% Pd/C; (c) *N*,*N*-dimethylthiocarbamoyl chloride, NaH, 39%; (d) diphenyl ether, 259 °C, 17 h, 82%; (e) KOH, ethanol, reflux; (f) MeOH, *p*-TsOH, reflux, steps (e–f) 40%.

order of increasing potency for the benzothiophene based compounds was *ortho* substitution, *meta*, and then *para*. The benzofuran based compounds closely matched their benzothiophene counterparts though they were generally less potent. The *meta* substituted benzyloxy-benzyl tails most closely resembled the aryl thiazole and isoxazole tails found in the benzofuran and benzothiophene examples in terms of shape, potency and selectivity. Interestingly, even though compounds with the *ortho* substituted benzyloxy-benzyl tails were less potent at PPARδ they had very little, if any, affinity for PPARα and PPARγ.

It is clear that there is a divergence between binding affinity and functional activity with many of these compounds.[10] This is explained by the lipophilicity of the compounds muting passive diffusion through the cell membrane in the cell-based functional assay. We

Table 2. Activity of indole based compounds



16a-g

Compound	X	R	Y = lipophilic tail	IC_{50}[a,c] (nM)			Fold selectivity: hPPARδ vs. hPPARα	EC_{50}[b,c] PPARδ (nM)
				hPPARδ	hPPARγ	hPPARα		
a	O	H	A	6.8	550	>10,000	81	223
b	O	H	C	31	835	>10,000	27	1390
c	O	5-MeO	A	41	121	NT	3	225
d	O	H	*o*-D	734	>10,000	>10,000	>14	3080
e	O	H	*m*-D	13	179	>10,000	14	254
f	O	H	*p*-D	91	562	>10,000	6	2810
g	S	H	A	2.8	172	>10,000	61	4.0

NT, not tested.

[a] Concentration that inhibits 50% of the interaction between the PPAR LBD and the radiolabeled ligand.

[b] Concentration of test compound which produced 50% of the maximal reporter activity.

[c] The results are based on at least two experiments, each dose done in triplicate (SD = 10%).

believed that the compounds were being sequestered within the cell membrane so that the observed functional activity was not always truly reflective of the compound's inherent potential. Nonetheless, the functional assay confirmed that all the compounds were agonist.

Starting with a prototypical PPAR ligand, applying a priori knowledge of the factors affecting subtype specificity, and a search of our proprietary chemical library, we discovered three new templates, that when modified had varying degrees of affinity and potency for PPARδ. These compounds represent a new class of chemical tools that will be used to elucidate the complete biological function of PPARδ.

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9. *PPAR receptor binding assay*: The human PPARδ and PPARα scintillation proximity assay (SPA) was used to measure the affinity of ligands for the respective human PPAR receptor. The hPPARδ LBD encoding amino acids 145–441 (GenBank Accession No. NM_006238) and hPPARα LBD encoding amino acids 196–468 (GenBank Accession No. L02932) were used. Volumes of 99 μL of buffer (50 mM Tris, 10 mM Na-molybdate, 1 mM EDTA, and 10% glycerol, pH 7.6) containing 50 nM of radiolabeled ligand (^{3}H-2-(4-(3-(4-acetyl-3-hydroxy-2 propylphenoxy)propoxy)phenoxy)acetic acid (34 Ci/mmol) for PPARδ and ^{3}H-2-(4-(2-(3-(2,4-difluorophenyl)-1-heptylureido)ethyl)phenoxy)-2-methylbutanoic acid (86 Ci/mmol) for PPARα), 0.2 mg anti-rabbit beads (Amersham, RPN140), 0.24 μg rabbit anti-GST (Molecular Probes Inc., A5800), and 0.2 μg purified GST/PPARhLBD were placed into the wells of Corning 96-well tissue culture plates. One microliter of dimethylsulfoxide (DMSO) or 1 μL of DMSO containing a test compound at a concentration sufficient to give a final assay concentration of between 1 nM and 100 μM added into each well. After incubation with shaking at room temperature for 30 min, radioactivity bound to the PPAR LBD-GST fusion protein/anti-GST/SPA antibody-binding bead complex was assessed using a Wallac MicroBeta plate reader. The potency of interaction of a compound with the respective PPAR LBD was determined as the concentration that inhibits 50% of the interaction between the respective PPAR LBD and the radiolabeled ligand.
The human PPARγ scintillation proximity assay (SPA) was used to measure the affinity of ligands for the human PPARγ receptor. The hPPARγ LBD encoding amino acids 206–477 (GenBank Accession No. NM_138712.1) was used. Volumes of 168 μL of buffer (1× PBS, 12 mM β-mercapto ethanol, 0.002% Tween 20, and 9% glycerol, pH 7.6) containing with 40 nM ^{3}H-5-(4-(3-(5-methyl-2-phenyloxazol-4-yl)propanoyl)benzyl)thiazolidine-2,4-dione (9.57 Ci/mmol), 0.3 mg polylysine-coated yttrium silicate beads (Amersham, RPNQ0010), and 10 nM purified His-tagged human PPARγ LBD were placed into the wells of a 96-well white assay plate (Corning 3604). Two microliters of dimethylsulfoxide (DMSO) or 2 μL of DMSO containing a test

compound at a concentration sufficient to give a final assay concentration binding curve between 1 nM and 100 μM was added into each well. After incubation with shaking at room temperature for 2 h, radioactivity bound to the PPARγ LBD-HIS fusion protein/yttrium bead complex was assessed using a Wallac MicroBeta plate reader. The potency of interaction of a compound with the PPARγ LBD was determined as the concentration that inhibits 50% of the interaction between the PPARγ LBD and the radiolabeled ligand.

10. *PPARδ chimeric receptor assay (functional assay): Transient transfection assay using the HepG2 hepatoma cell line:* The GAL4 hPPARδ LBD, chimeric receptor expression constructs containing the ligand binding domain for the human PPARδ LBD (encoding amino acids 145–441, GenBank Accession No. NM_006238), was used to cotransfect cells with GAL4-Luciferase reporter plasmid (p5Eb-Luc) and β-Gal plasmid. Briefly, HepG2 cells were seeded in a 100-mm cell culture dish containing 10 ml DMEM plus 10% serum. Transfection mix was prepared by combining 15 μg GAL4-Luc plasmid with 15 μg of GAL4-hPPARδ LBD. β-Gal plasmid (1.5 μg) was also added to each the as a control. LipofectAMINE 2000 reagent was used as suggested by the manufacturer (Invitrogen, Carlsbad, CA). For each well, 2.4 ml transfection mix was added and incubated at 37 °C overnight. The next day, transfected HepG2 cells were reseeded to a 96-well cell culture plate at the density of 3000 cells per well and compounds were subsequently added to each well. After 16 h incubation, cells were then harvested in a lysis buffer (Promega, Madison, Wisconsin) and luciferase activity was determined using a luminometer. Luciferase activity was then normalized with β-Gal activity.
11. Typical procedure for preparing thiazolyl benzothiophene acetic acid derivatives:*4-(3-Methoxy-phenylsulfanyl)-3-oxo-butyric acid methyl ester* **(2)**. A solution of methyl 2-chloroacetoacetate (15.0 g, 0.10 mol) in 20 ml of acetonitrile was added dropwise to a mixture of 3-methoxythiophenol (14.0 g, 0.10 mol) and cesium carbonate (65.2 g, 0.20 mol) in 400 ml of acetonitrile over 30 min. The mixture was stirred at room temperature for 2 h, then filtered through Celite®. The filtrate was concentrated and purified using normal phase chromatography.

(6-methoxybenzo[b]thiophen-3-yl)acetic acid methyl ester **(3)**. Compound **2** (2.54 g, 0.01 mol) was added dropwise to 25 ml of methanesulfonic acid at room temperature, and the solution was stirred at the same temperature for 15 min, then the reaction mixture was added to 250 ml of ice-water. The aqueous mixture was extracted with ethyl acetate. The organic phase was washed with brine, sodium bicarbonate, dried over sodium sulfate, and concentrated.

(6-hydroxy-benzo[b]thiophen-3-yl)acetic acid methyl ester **(6).** To a stirred solution of **3** (2.20 g, 9.32 mmol) in 50 ml of dichloromethane at −78 °C was added dropwise a solution of boron tribromide (11.68 g, 46.6 mmol) in 50 ml of dichloromethane. After the completion of the addition of boron tribromide, the reaction mixture was maintained at −78 °C for 1 h, then allowed to reach room temperature and stirred at the same temperature overnight. The mixture was cooled to 0 °C, carefully quenched with 100 ml of water, extracted with ethyl acetate, washed with brine, dried over sodium sulfate, concentrated, and purified using normal phase chromatography to afford **6**.

Methyl 2-(6-((4-methyl-2-(4-(trifluoromethyl) phenyl)thiazol-5-yl)methoxy)benzo[b]thiophen-3-yl)acetate **9a**. A mixture of **6** (0.75 g, 3.4 mmol), 5-(chloromethyl)-4-methyl-2-(4-(trifluoromethyl) phenyl)thiazole (1.0 g, 3.5 mmol), and cesium carbonate (1.7 g, 5.1 mmol) in 10 ml acetonitrile was stirred at 60 °C for 4 h. The reaction mixture was then concentrated in vacuo, the residue diluted with ether and filtered through a Celite®/SiO_2 sandwich, eluting with ether. The filtrate was collected and concentrated in vacuo to give **9a** pure enough for subsequent use.

2-(6-((4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5- yl)-methoxy)benzo[b]thiophen-3-yl)acetic acid **(10a)**. A mixture of **9a,** in 40 ml THF/2 ml H_2O, was stirred at rt for 2 h. The reaction mixture was concentrated in vacuo, acidified to pH 1 with concd HCl, extracted with EtOAc, dried (Na_2SO_4), and concentrated in vacuo. Recrystalization from chloroform/hexanes afforded **10a**.