Structure-Based Optimization of Protein Tyrosine Phosphatase 1B Inhibitors: From the Active Site to the Second Phosphotyrosine Binding Site

Douglas P. Wilson,[†] Zhao-Kui Wan,*[,†] Wei-Xin Xu,[†] Steven J. Kirincich,[†] Bruce C. Follows,[†] Diane Joseph-McCarthy,[†] Kenneth Foreman,[†,‡] Alessandro Moretto,[†] Junjun Wu,[†] Min Zhu,[†] Eva Binnun,[†] Yan-Ling Zhang,[§,||] May Tam,[§] David V. Erbe,[§] James Tobin,[§] Xin Xu,[⊥] Louis Leung,[∞] Adam Shilling,[∞] Steve Y. Tam,[†] Tarek S. Mansour,[†,▽] and Jinbo Lee[†,#]

Chemical and Screening Sciences, and Cardiovascular and Metabolic Diseases, Wyeth Research, 200 Cambridge Park Drive, Cambridge, Massachusetts 02140, Chemical and Screening Sciences, Wyeth Research, 401 Middletown Road, Pearl River, New York 10965, Discovery PK, Wyeth Research, One Burtt Rd, Andover, Massachusetts 01810, and Biotransformation, Wyeth Research, 500 Arcola Road, Collegeville, Pennsylvania 19426

Received March 5, 2007

Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of the insulin and leptin receptor pathways and thus an attractive therapeutic target for diabetes and obesity. Starting with a high micromolar lead compound, structure-based optimization of novel PTP1B inhibitors by extension of the molecule from the enzyme active site into the second phosphotyrosine binding site is described. Medicinal chemistry, guided by X-ray complex structure and molecular modeling, has yielded low nanomolar PTP1B inhibitors in an efficient manner. Compounds from this chemical series were found to be actively transported into hepatocytes. This active uptake into target tissues could be one of the possible avenues to overcome the poor membrane permeability of PTP1B inhibitors.

1. Introduction

Protein tyrosine phosphatase 1B (PTP1B[a]) is a negative regulator of the insulin and the leptin receptor pathways.[1−4] Two independent studies of PTP1B-deficient mice have revealed phenotypes of enhanced insulin sensitivity, improved glycemic control, and resistance to high fat diet induced obesity.[5,6] Furthermore, treatment of diabetic mice with PTP1B antisense oligonucleotides reduced the expression level of the enzyme and subsequently normalized blood glucose and improved insulin sensitivity.[7,8] Thus PTP1B has been considered as an attractive therapeutic target for type 2 diabetes and obesity.[9−11] Small molecule inhibitors of this enzyme have been pursued extensively by both industry and academia.[12−21] However, compounds that have good potency and are readily accessible to target tissues remain elusive.[22,23]

Our PTP1B small molecule inhibitor program started with a weak but well characterized lead compound, **1**, which binds to the enzyme active site (site A) and has a K_i value of 230 μM (Figure 1).[24] Early exploration of the inhibitor scaffolds resulted in the discovery of a tricyclic compound, **2** ($K_i = 9.2$ μM), which was ∼25 fold more potent than **1**.[24] This result suggested that the bicyclic thienopyridine template was not required for enzyme inhibition. Upon further exploration, it was later discovered that the pyridyl ring could be completely removed and resulted in the design and synthesis of **3**, a compound that was 70-fold more potent than our starting lead **1**.[25] An X-ray cocrystal structure of PTP1B and **3** revealed that the 3′-position was pointing toward the second phosphotyrosine binding site (site B), a site that has been shown to offer opportunities for both potency and selectivity improvement.[26,27] Attempts to bridge a fragment for site A and a fragment for site B using flexible linkers have produce mixed results.[28] Although notable success was achieved by scientists from Abbott Laboratories,[16,29] the use of flexible linkers may have incurred a high degree of rotational freedom. This in turn may be responsible for the poor physical−chemical properties for the overall molecules. Our approach was to build our PTP1B inhibitors from site A toward site B using rigid functionality in a stepwise manner, in which atom efficiency could be optimized along the way as guided by X-ray complex structures. Our structure-based effort resulted in significant improvements in potency and selectivity and is discussed herein.

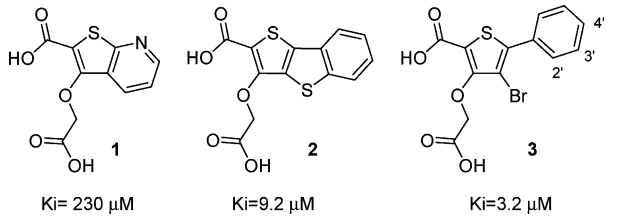


Figure 1. Comparison of bicyclic, tricyclic, and monocyclic thiophenes as PTP1B inhibitors.

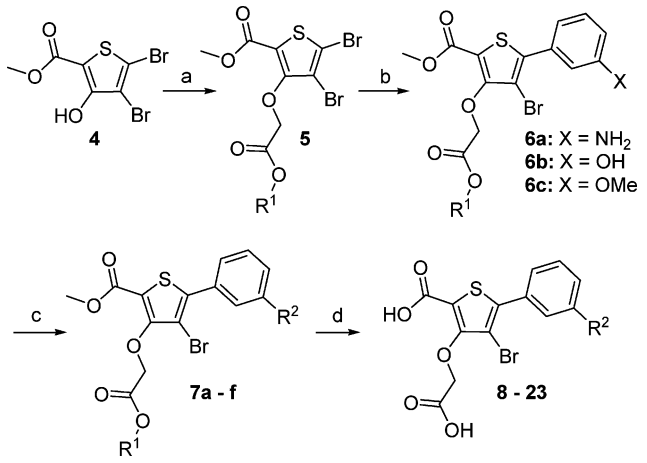
2. Chemistry

The synthesis of PTP1B inhibitors started with alkylation of **4** with ethyl bromoacetate or *tert*-butyl bromoacetate, followed by Suzuki coupling with various boronic acids to provide **6a**−**c** in good yields (Scheme 1). The Suzuki coupling reaction was highly regioselective toward the C5-position when $Pd(PPh_3)_4$ was used as the catalyst and THF was used as the solvent in the reactions.[25] Intermediate **6a** was further derivatized according to standard procedures to give various amides, ureas, sulfonamides, carbamates, and alkylamino analogs **7**.[30−32] Intermediate

* Corresponding author. Phone: 617-665-5635. Fax: 617-665-5682. E-mail: zwan@wyeth.com.
[†] Chemical and Screening Sciences, Wyeth Research, Cambridge.
[‡] Present address: OSI Pharmaceuticals, Inc., 1 Bioscience Park Dr., Farmingdale, NY 11735.
[§] Cardiovascular and Metabolic Diseases, Wyeth Research, Cambridge.
[||] Present address: Merck Research Laboratories, 33 Avenue Louis Pasteur, Boston, MA 02115.
[⊥] Discovery PK, Wyeth Research, Andover.
[∞] Biotransformation, Wyeth Research, Collegeville.
[▽] Chemical and Screening Sciences, Wyeth Research, Pearl River.
[#] Present address: Ensemble Discovery Corp., 99 Erie St., Cambridge, MA 02139.
[a] Abbreviations: PTP1B, protein tyrosine phosphatase 1B; TCPTP, T-cell protein tyrosine phosphatase 1B.

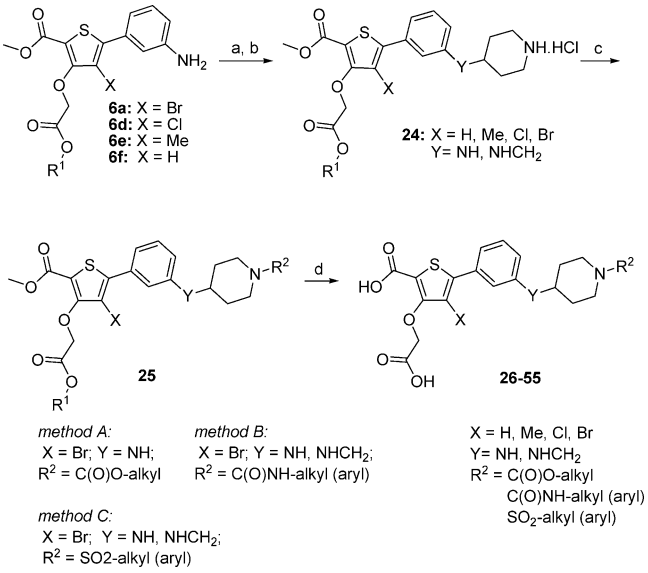
10.1021/jm0702478 CCC: $37.00
Published on Web 08/17/2007

Scheme 1. Synthesis of Compounds **8**−**23**



Reagents and conditions: (a) ethyl (or *tert*-butyl) bromoacetate, K_2CO_3, DMF, 60 °C (R_1 = Et or *t*Bu); (b) $Pd(PPh_3)_4$, arylboronic acid, KF, THF, 100 °C; (c) X = NH_2, (i) acid chloride (isocyanate, chloroformate, or sulfonyl chloride), DIPEA, CH_2Cl_2, rt; (ii) ketone (or aldehyde), $NaBH(OAc)_3$, HOAc, DCE, rt; X = OH, benzyl bromide, K_2CO_3, DMF, 60 °C; (d) $LiOH{\cdot}H_2O$, THF/H_2O, rt.

Scheme 2. Synthesis of Compounds **26**−**55**

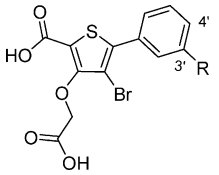


Reagents and conditions: (a) *tert*-butyl 4-oxopiperidine-1-carboxylate (or *tert*-butyl 4-formylpiperidine-1-carboxylate), $NaBH(OAc)_3$, AcOH, DCE, rt; (b) HCl/EtOAc/MeOH, rt; (c) method A, chloroformate, pyridine, rt; method B, isocyanate, DMF, rt; method C, sulfonyl chloride, pyridine, rt; (d) $LiOH{\cdot}H_2O$, THF/H_2O, rt.

6b was alkylated with benzyl bromide to give the precursor of compound **17**. Hydrolysis of **6a**−**c** and **7** using LiOH in THF/water provided the compounds **8**−**23** in good to excellent yields.

Compounds **26**−**55** were prepared by following the reaction steps in Scheme 2. The synthesis started with intermediate **6a** or **6d**−**f**, which were prepared according to the procedures in Scheme 1. In the synthesis of **6d**, methyl 4,5-dichloro-3-hydroxythiophene-2-carboxylate was prepared by following literature procedures[33] and was subsequently used in the Suzuki coupling reaction. Reductive amination of **6a** (or **6d**−**f**) and *tert*-butyl 4-oxopiperidine-1-carboxylate (or *tert*-butyl 4-formylpiperidine-1-carboxylate) gave intermediate **24**. Deprotection with TFA in CH_2Cl_2 and subsequent reactions with acid chlorides, chloroformates, isocyanates, or sulfonyl chlorides in the presence of DIPEA provided intermediates **25** in good to excellent yields.

Table 1. Inhibition Constants of Compounds **8**−**17** against PTP1B Enzyme



compd	R	K_i (μM)
8	NH_2	1.7
9	OH	1.0
10	OMe	4.0
11	$NHC(O)CH_2CH_3$	2.0
12	NHC(O)Ph	2.1
13	$NHC(O)NHCH(CH_3)_2$	2.1
14	$NHC(O)OCH_3$	2.0
15	$NHS(O)_2$(4-F-Ph)	0.44
16	NH-benzyl	0.47
17	O-benzyl	0.68

Final deprotection using LiOH in THF/water completed the synthesis of compounds **26**−**55**.

3. Results and Discussion

3.1. Structure−**Activity Relationship**. The 3′-position of **3** offers a favorable trajectory toward the second phosphotyrosine binding site, as revealed by previously reported X-ray complexes of **3** and PTP1B.[25] To effect the extension of the molecule from the active site toward the second phosphotyrosine binding site, an amino or a hydroxyl group was introduced as a functional handle. Compared to compound **3**, a hydrogen bond donor such as an amino (**8**) or a hydroxyl group (**9**) at the 3′ position provided slight improvements in potency, 2−3-fold, respectively (Table 1). Removal of the hydrogen-bond donor on compound **9** and replacing it with a methoxy group (**10**) caused a 4-fold decrease in inhibitory activity. Furthermore, attempts to increase the acidity of the NH on compound **8** using amides (e.g., **11**, **12**), ureas (e.g., **13**), and carbamates (e.g., **14**) did not translate into better inhibitory activity. The structure−activity relationship remained flat and the K_i of these compounds remained around 2 μM. These results suggested that a hydrogen-bond donor, presumably interacting with Asp48, might be favorable at the 3′-position. However, the directionality of the hydrogen bond was not as optimal as that between a hydrogen-bond donor (e.g., OH or NH) at the 4′-position and the Asp48 residue, which has been reported previously.[25]

Some sulfonamide analogs did provide modest improvements in potency (e.g., **15** vs **8**). However, it was not clear whether the acidity of the NH group or the hydrogen-bonding played a significant role because the NH-benzyl (**16**) or O-benzyl (**17**) groups offered similar improvement in inhibitory activity. In the case of compound **17**, there is no hydrogen-bond donor at the 3′-position. Thus, van der Waals interactions between the inhibitors and the enzyme might be more important. The X-ray complex structure of **16** and PTP1B provided the first hint of our success in extending the inhibitor into the second phosphotyrosine-binding site (Figure 2). The benzyl side chain of **16** has passed through the "gateway" between the active site and the second phosphotyroine binding site, which is lined with the Gly259 residue. Consistent with the structure−activity relationship (SAR) in Table 1, the benzyl group of **16** bends toward Met258 and forms a van der Waals contact with its side chain. This is the key contributing factor for the potency improvement in the case of **16** and **17** (the X-ray complex structure of **17** and PTP1B has also been solved, but is not shown here; the benzyl group of **17** overlays nicely with that of **16**).

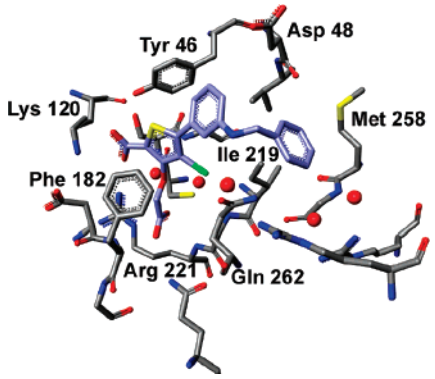
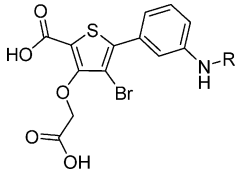


Figure 2. X-ray complex structure of **16** and PTP1B. The inhibitor (colored by element with carbons in purple) and key active site residues (colored by element with carbons in gray) are shown in a capped stick representation.

Table 2. Inhibition Constants of Compounds **18**−**23** against PTP1B Enzyme



compd	R	K_i (μM)
18	cyclohexylmethyl	0.20
19	cyclohexyl	0.21
20	cyclopentyl	0.39
21	cycloheptyl	0.12
22	4-tetrahydro-2*H*-pyranyl	0.31
23	3,3,5,5-tetramethylcyclohexyl	0.036

The SAR at the 3′-position has been expanded to include a variety of cycloalkyl groups (Table 2), in order to optimize van der Waals interaction with the Met258 side chain. Indeed, unsaturated cycloalkyl groups (**18**−**21**) also increased inhibitory activity significantly, with K_i values 2−4-fold better than that of the corresponding benzyl analog (**16**). Cyclohexylmethyl (**18**) and cyclohexyl (**19**) analogs have similar potency ($K_i \sim 200$ nM), but the latter have better atom efficiency due to the lack of a methylene spacer. Incorporation of an oxygen atom on the cyclohexyl ring as in the case of compound **22** resulted in little change in potency against the PTP1B enzyme.

The X-ray complex structure of **19** and PTP1B revealed the orientation of the cyclohexyl ring, which lies flat along the tunnel wall between the active site and the second phosphotyrosine binding site (Figure 3A). The side chain of Arg24 adopts a folded-down conformation to line the outer wall of this tunnel. In contrast, the Arg24 side chain generally adopts a fold-up conformation in the apo protein[34,35] and in the complex structures of inhibitors without a side chain into the second phosphotyrosine binding site (e.g., **3** and PTP1B).[25] Consistent with that of compound **16** (Figure 2), van der Waals interactions between the cyclohexyl group of **19** and the Met258 side chain play an important role in binding affinity. In addition, the cocrystal structure revealed opportunities for further improvement of these van der Waals interactions (Figure 3A). There is a small hydrophobic "hole" next to Met258, a residue against which the cyclohexyl side chain of **19** packs. Molecular modeling suggested that a methyl group extending out from the 3″-position of the cyclohexyl ring could provide a good fit into this hydrophobic cavity (Figure 3A,B). To avoid introducing chirality into the molecule, compound **23** was designed and synthesized with a symmetric 3,3,5,5-tetramethylcyclohexy-

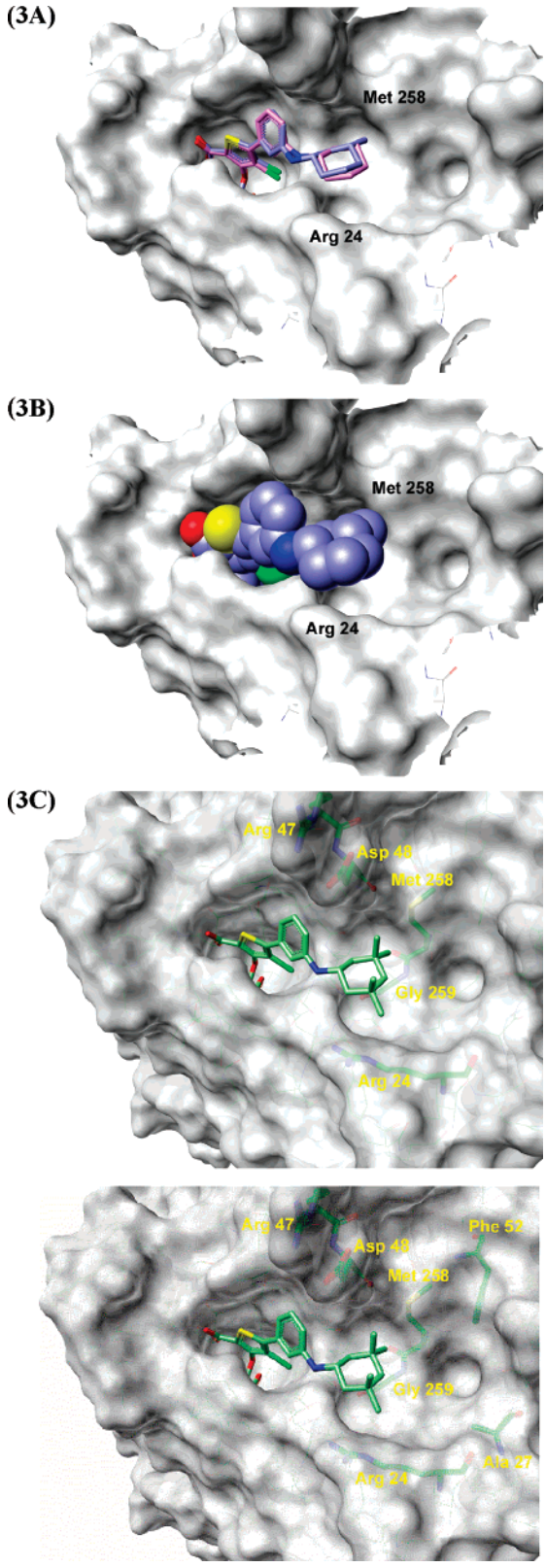
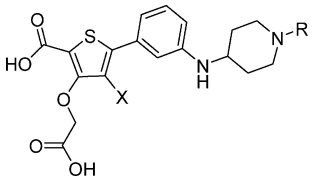


Figure 3. (A) X-ray structure of **19** (carbons in pink) bound to PTP1B superimposed with a model for a compound with an additional methyl (carbons in purple) bound to PTP1B. The small molecules are shown in capped stick representation, and a molecular surface is shown on the protein in gray. (B) Model of the compound with the additional methyl bound to PTP1B. A molecular surface is shown on the protein in gray with the ligand in a space-filling model colored by element with carbons in purple. (C) X-ray structure of **23** bound to PTP1B with a transparent molecular surface in gray on the protein. The inhibitor and key active site residues (colored by element with carbons in green) are shown in a capped stick representation.

Table 3. Inhibition Constants of Compounds **26**−**35** against PTP1B Enzyme

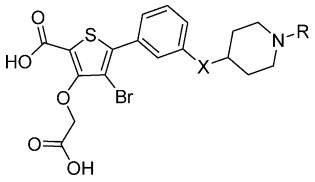


compd	X	R	K_i (μM)
26	Br	H	3.8
27	Br	$C(O)CH_3$	0.19
28	Br	$C(O)OCH_3$	0.39
29	Br	$C(O)NHCH_2Ph$	0.30
30	Br	$S(O)_2CH_3$	0.044
31	Br	$S(O)_2Ph$	0.055
32	Br	$S(O)_2CH_2Ph$	0.004
33	H	$S(O)_2CH_2Ph$	2.0
34	Me	$S(O)_2CH_2Ph$	0.008
35	Cl	$S(O)_2CH_2Ph$	0.00068

lamino group introduced at the 3′-position of **3**. Confirming the molecular modeling prediction, the presence of the methyl groups at the 3″-position of the cyclohexyl ring significantly improved inhibitory activity against PTP1B. Compound **23** has a K_i of 36 nM, almost an order of magnitude better than that of **19**. More interestingly, the binding mode of **23** to PTP1B was exactly as predicted by molecular modeling, with a methyl group nicely filling the hydrophobic "hole" next to Met258 side chain (Figure 3C).

The design and synthesis of **23** was inspired by structural information from X-ray complexes. Though a potent and efficient inhibitor of PTP1B enzyme, compound **23** does not fully occupy the second phosphotyrosine binding site (Figure 3C). Its 3,3,5,5-tetramethylcyclohexyl side chain is still some distance from Phe52 and Ala27, the residues that are situated at the far end of this site. These residues are different in PTP1B vs in T-cell protein tyrosine phosphatase (TCPTP),[27,36] which may offer an opportunity for selectivity. To extend the molecule further into the second phosphotyrosine binding site, an aminopiperidine group was introduced at the 3′-position of **3** as a functional handle (Scheme 2). Compound **26** (K_i = 3.8 μM), with the piperidine group, was much less active than **19** (K_i = 0.21 μM), which might due to the proximity of its basic nitrogen to residues Arg24 and Arg254 (Table 3). Neutralizing the basic nitrogen of **26** with amide (e.g., **27**), carbamate (e.g., **28**), and urea (e.g., **29**) recovered the inhibitory potency against PTP1B ($K_i \sim 0.2-0.3$ μM). What really stood out among the various modifications was the methylsulfonamide analog **30**, with a K_i of 44 nM, even though it lacked the methyl groups of compound **23**. Replacing the methylsulfonamide (**30**) with a phenylsulfonamide (**31**) did not offer any added benefit. However, inserting a methylene spacer between the sulfonyl group and phenyl group resulted in a significant improvement in potency. Compound **32**, the first single digit nanomolar compound in this chemical series (K_i = 4 nM) was thus obtained. The potency of this chemical series could be further improved by fine-tuning the substitution at the 4-position of the thiophene template, which presumably adjusts the positioning of the benzyl sulfonamide side chain in the second phosphotyrosine binding site. The attempt to synthesize a tetramethylpiperidine sulfonamide was not successful. Replacing the bromo group (**32**) with a hydrogen (**33**) also resulted in significant loss in potency (almost 3 orders of magnitude). While a methyl group (**34**) was tolerated here, a chloro group (**35**) provided the best potency with subnanomolar activity against PTP1B (K_i = 0.68 nM, Table 3). In general, replacing the 4-bromo with a 4-chloro group

Table 4. Inhibition Constants of Compounds **36**−**40** against PTP1B Enzyme



compd	X	R	K_i (μM)
36	O	$S(O)_2CH_2Ph$	0.024
37	$NHCH_2$	$S(O)_2CH_2Ph$	0.074
38	$NHCH_2$	$S(O)_2Ph$	0.31
39	$NHCH_2$	C(O)NHPh	0.007
40	OCH_2	C(O)NHPh	0.013

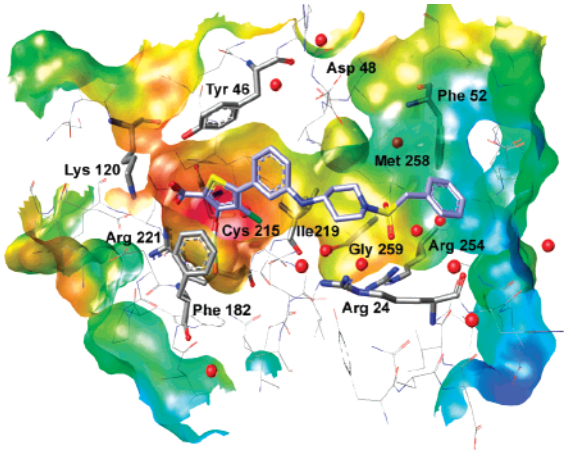


Figure 4. X-ray structure of **32** bound to PTP1B shown with molecular surface on the protein colored by electrostatic potential. The inhibitor (colored by element with carbons in purple) and key active site residues (colored by element with carbons in gray) are shown in a capped stick representation.

offered about 4−6-fold improvement in potency (analogs of **23** showed the similar trend; data not shown). Compared to the original lead (**1**), this structure-based optimization effort produced a compound (**35**) that is >300 000-fold more potent.

Fine-tuning the linker region of **32** was also attempted with representative examples shown in Table 4. Replacing the NH linker (**32**) with an oxygen atom (**36**) at the 3′-position resulted in a slight loss of potency (~6-fold). Inserting a methylene between the NH and the piperidine benzyl sulfonamide (**37**) was not favorable. In the case of compound **37**, shortening the side chain by removing the methylene on the sulfonamide side (**38**) resulted in further loss of activity. Surprisingly, replacing the sulfonamide side chains with a phenyl urea group (**39**) regained single-digit nanomolar potency. The NH linker of **39** could be interchanged with an oxygen linker (**40**) without a significant loss in potency (Table 4).

The X-ray complex structure of **32** and PTP1B clearly indicates how this compound interacts with the enzyme (Figure 4). Compound **32** fully occupies both the active site and the second phosphotyrosine binding site, with the thiophene head group at the active site and the benzylsulfonamide group at the second site. One of the sulfonyl oxygens forms a hydrogen bond with a backbone amide, and the other oxygen interacts with Arg24 and Arg254 via water-mediated hydrogen bonds. These hydrogen bond interactions may compensate for the loss of the van der Waals interaction between the methyl group of **23** and Met258. This likely explains why compounds **30** and **23** have similar potency. The benzyl group caps two water molecules at the second phosphotyroine binding site and interacts with

Table 5. Inhibition Constant of Compounds **3**, **23**, and **32** against PTP1B, CD45, LAR, and TCPTP

	K_i (μM)			
compd	PTP1B	CD45	LAR	TCPTP
3	3.2	280	>500	1.3
23	0.036	151	>1,000	0.026
32	0.004	77	>500	0.005

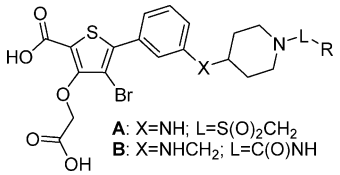
Phe52 in a edge-to-edge fashion. All these interactions combined in an additive fashion result in the high affinity of **32** to PTP1B enzyme.

3.2. Selectivity against Other Phosphatases. In addition to potency improvements, one added benefit for extending PTP1B inhibitors from the active site into the second phosphotyrosine binding site could be improving selectivity against other protein tyrosine phosphatases (PTPases). Assay results of three representative compounds, **3**, **23**, and **32**, against four different phosphatases (PTP1B, CD45, LAR, and TCPTP) are shown in Table 5. Compound **3**, which resides only at the active site, has excellent selectivity against LAR (>150 fold), good selectivity against CD45 (~87 fold), but is more active against TCPTP (~2−3-fold more active against TCPTP vs PTP1B). Compound **23**, the side chain of which has entered the second phosphotyrosine binding site, improves selectivity against CD45 (~4200-fold). The improved selectivity between PTP1B and CD45 may be partially due to the differences at the gateway between the active site and the second phosphotyrosine binding site of these two enzymes.[26] PTP1B has a glycine (Gly259) lining the bottom of the gateway, while CD45 has the bigger amino acid, leucine. In addition to steric hindrance at the gateway, other interactions between the inhibitor and the second phosphotyrosine binding site may also contribute to selectivity. Compound **32**, which fully occupies both the active site and the second phosphotyrosine binding site, shows the best selectivity against CD45 (~19 000-fold). Furthermore, compound **32** has shifted the relative selectivity between PTP1B and TCPTP when compared to compound **3** (Table 5).

To further probe the second phosphotyrosine binding site and the relative selectivity between PTP1B and TCPTP, various substitutions on the phenyl groups of **32** (series A) and **39** (series B) were examined. Some representative examples are shown in Table 6. In general, the potency and relative selectivity of these analogs has the following order: ortho > meta > para (e.g., **41** vs **42** vs **43**). This is consistent with the X-ray complex structure of **32** and PTP1B (Figure 4), in which the para-position of the benzyl group packs tightly against the protein surface and thus does not allow further substitution. Therefore, effort was concentrated on exploring the effect of ortho-substituents on potency and relative selectivity between PTP1B and TCPTP. For analogs of **32** (series A), various substituents are tolerated (**44**−**50**). The best potency (~1 nM) and relative selectivity (~2-fold) was seen in compound **50**, with a methylsulfonamide group at the ortho-position. Analogs of **39** (series B) show a similar trend (ortho > meta > para, data not shown). Again, relative selectivity between PTP1B and TCPTP ranges from 2- to 3-fold (e.g., **51**−**54**). In conclusion, extending the PTP1B inhibitor into the second phosphotyrosine binding site does improve selectivity against other PTPases (e.g., CD45). However, the potential of this site for significantly distinguishing PTP1B from TCPTP remains a challenge, due to the high homology of these two enzymes.[15]

3.3. Physical−Chemical Properties, Metabolic Stability, and Phamacokinetics. The physical−chemical properties of compound **32** have been evaluated extensively. This compound has good aqueous solubility (>100 μg/mL at pH 7.4 buffer). However, permeability across biological membranes is limited due to its polar head group. Its Caco-2 permeability coefficient (Pe) is less than 1×10^{-6} cm/s. The stability of **32** in liver microsomes has been profiled across three different species. It has good stability in human ($T_{1/2}$ >60 min), mouse ($T_{1/2}$ >60 min), and rat ($T_{1/2}$ ~46 min) liver microsomes. Furthermore, compound **32** shows little inhibition of various cytochrome P450 enzymes (IC_{50} > 500 μM for CYP1A2, CYP2D6, CYP3A4, CYP2A6, CYP2C8, CYP2C19, and 40 μM for CYP2C9). Therefore, there appears to be little concern about potential clinical drug−drug interactions for this compound.

Table 6. Inhibition Constant of Compounds **41**−**55** against PTP1B and TCPTP



			K_i (μM)	
compd	series	R	PTP1B	TCPTP
41	A	4-Cl-Ph	0.038	0.010
42	A	3-Cl-Ph	0.010	0.009
43	A	2-Cl-Ph	0.005	0.004
44	A	2-CF_3-Ph	0.006	0.002
45	A	2-Me-Ph	0.004	0.006
46	A	2,6-di-Me-Ph	0.006	0.008
47	A	2-NH_2-Ph	0.002	0.002
48	A	2-NHC(O)Me-Ph	0.002	0.002
49	A	2-NHC(O)NHEt-Ph	0.014	0.012
50	A	2-$NHSO_2$Me-Ph	0.001	0.002
51	B	2-Cl-Ph	0.004	0.006
52	B	2-OMe-Ph	0.003	0.005
53	B	2-Me-Ph	0.003	0.005
54	B	2,6-di-Me-Ph	0.003	0.009

Table 7. Concentration of **32** in Plasma, Liver, and Muscle Tissues from PK Studies (10 mg/kg, ip)

	concn (μM)		
time (h)	plasma	liver	muscle
1	15.2[a]	6.9	3.5
7	0.37	0.17	0.26

[a] C_{max}.

The pharmacokinetics (PK) of **32** has been examined in two species: male Sprague−Dawley rats and C57/B6 mice. In rats receiving a single iv (intravenous) bolus injection of 2 mg/kg, compound **32** has a low plasma clearance (CL ~ 7 mL/min/kg) and a long elimination half-life ($t_{1/2}$~ 10 h). This is in good agreement with the excellent in vitro stability of this compound in liver microsomes. Further PK studies were carried out in mice where compound **32** was administered into mice at 10 mg/kg via ip (intraperitoneal) injection. Compound concentrations in plasma were evaluated at various time points, and the tissue concentrations in liver and muscle were measured at 1 and 7 h after ip injection (Table 7). The C_{max} of **32** in plasma was observed at 1 h after ip administration, with a mean concentration of 15.2 μM ($N = 3$). Surprisingly, a substantial amount of **32** was observed in both liver and muscle tissues at the 1 h time point, despite the low membrane permeability of this compound. The corresponding mean ± SD concentration in the liver and muscle was 6.9 ± 2.1 and 3.5 ± 2.7 μM, respectively. At the 7 h time point, compound concentrations deceased significantly in all tissues including plasma to a range of 0.2−0.4 μM.

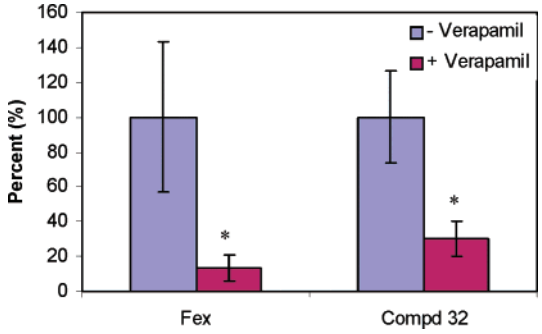


Figure 5. Uptake of **32** and fexofenadine into rat hepatocytes in the presence and absence of verapamil. Significant differences in uptake in the presence of verapamil ($p < 0.05$, *t*-test) are denoted with "*".

3.4. Cellular Uptake. Compound **32** showed low membrane permeability in the Caco2 assay and thus was expected to have low accessibility to target tissues such as liver and muscle in vivo. However, a significant amount of **32** was detected in liver tissue during the ip PK study in C57/B6 mice (Table 7). This surprising observation was suggestive of a possible active uptake mechanism. Since compound **32** exists as an anion under physiological conditions, it was suspected that one or more organic anion transporting polypeptides (OATPs) may be involved in its delivery into liver tissues. To evaluate this possibility, an experiment was performed to study the uptake of **32** into primary rat hepatocytes in the absence and presence of an OATP inhibitor, verapamil.[37] Fexofenadine, a known substrate of OATPs, was used as a positive control in this experiment.[37,38] The results of this study are summarized in Figure 5. The OATP inhibitor, verapamil, caused an 87% decrease in the uptake of fexofenadine into hepatocytes. Comparably, a 70% decrease in the uptake of **32** was observed in the presence of verapamil. This supports the hypothesis that **32** is a substrate of organic anion transporting polypeptides and is indeed actively transported into hepatocytes. Thus, the high concentration of **32** in liver tissues during PK studies could be attributed to its active uptake. In vitro systems such as OATP-expressing or transfected cells (e.g., HeLa, MDCK or *Xenopus laevis* ooctyes)[39] may help to further assess the involvement of different rat (OATP1, 2 or 4) or human (OATP1B1, 1B3 or 2B1) OATPs in the hepatic uptake of **32** or analogs. Results of in vivo experiments following ip dosing in ob/ob mice were not reproducible and therefore cannot be used as an in vivo proof of concept. Further work on the use of prodrugs of these potent inhibitors for demonstration of in vivo efficacy will be reported separately.

4. Conclusion

PTP1B is an attractive therapeutic target for type 2 diabetes and obesity. The nature of its active site, which favors highly polar molecules, has created difficulties for drug discovery. The present report demonstrates a successful use of structural information in guiding the extension of PTP1B inhibitors from the active site to the second phosphotyrosine binding site to improve potency and selectivity in an efficient manner. A more than 300 000-fold improvement in potency was achieved using this approach (**1** vs **32**). Furthermore, careful examination of PK data led to the discovery of an active uptake mechanism of this class of compounds (e.g., **32**) into hepatocytes. This could be a possible avenue to overcome the low passive diffusion and thus poor tissue accessibility of polar PTP1B inhibitors. Future effort in further improving the physical−chemical properties of these PTP1B inhibitors for better delivery in vivo will be presented in due course.

5. Experimental Section

5.1. Chemistry. Commercial reagents and solvents were used as received without further purification. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer. LC−MS data were collected using a Micromass LCT mass spectrometer with electrospray ionization in conjunction with a Waters 2795 LC system. Liquid chromatography (LC−MS) was performed using a Phenomenex C18 column (Mercury MS Luna 5μ C18(2), 20 × 2 mm) with mobile phase of 0.1% formic acid (FA) in H_2O (A) and 0.1% formic acid in MeCN (B) and a gradient of 15−100% B in 3 min followed by 1.5 min at 100% B. HRMS data were recorded on a Bruker APEXIII-7T FTMS spectrometer with electrospray ionization. Purity of compounds was also analyzed by RF-HPLC using the following two conditions: (1) H_2O/MeCN/0.1% FA; (2) H_2O/MeOH/0.1% FA (a gradient of 5−100% B in 7 min followed by 2 min at 100% B).

4,5-Dibromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic Acid Methyl Ester (5). General Procedure of Alkylation. Potassium carbonate (13.07 g, 94.7 mmol) and ethyl bromoacetate (10.5 mL, 94.7 mmol) were added to a 150 mL of a DMF solution of 4,5-dibromo-3-hydroxythiophene-2-carboxylic acid methyl ester (20 g, 63.1 mmol). The resulting suspension was stirred at 50 °C overnight. The reaction mixture was cooled to room temperature, followed by addition of 200 mL of Et_2O. The mixture was filtered through a pad of Celite to remove any solid materials. Solvents were evaporated under reduced pressure, and the resulting solid was dissolved in CH_2Cl_2 and loaded onto a pad of silica. The crude product was purified by flash column chromatography using 1/6 EtOAc/hexane as eluent. 4,5-Dibromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (24.9 g, 99%) was obtained as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.30 (t, $J = 7.07$ Hz, 3 H), 3.85 (s, 3 H), 4.27 (q, $J = 7.07$ Hz, 2 H), 4.90 (s, 2 H).

4,5-Dibromo-3-*tert*-butoxycarbonylmethoxylthiophene-2-carboxylic acid methyl ester (4.31 g, 95%) was prepared from 4,5-dibromo-3-hydroxythiophene-2-carboxylic acid methyl ester (3.17 g, 10 mmol), *tert*-butyl bromoacetate (1.77 mL, 12 mmol), and K_2CO_3 (2.76 g, 20 mmol) according to the same alkylation procedure. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.42 (s, 9 H), 3.78 (s, 3 H), 4.75 (s, 2 H). Anal. Calcd for $C_{12}H_{14}Br_2O_5S$: C, 33.51; H, 3.28; N, 0. Found: C, 33.60; H, 3.08; N, 0.

5-(3-Aminophenyl)-4-bromo-3-*tert*-butoxycarbonylmethoxythiophene-2-carboxylic Acid Methyl Ester (6a). General Procedure for Suzuki Coupling Reaction. 4,5-Dibromo-3-*tert*-butoxycarbonylmethoxylthiophene-2-carboxylic acid methyl ester (130 mg, 0.3 mmol), 3-aminophenylboronic acid (66 mg, 0.42 mmol), KF (52.2 mg, 0.9 mmol), and $Pd(PPh_3)_4$ (17.3 mg, 0.015 mmol) in THF (4 mL) were mixed in a sealed tube. The reaction mixture was heated at 120 °C in a microwave reactor (Personal Chemistry) for 20 min. The precipitate was removed by filtering through a pad of Celite and then washed with EtOAc. The crude product was purified by flash column chromatography using 1/10 EtOAc/hexane as eluent. 5-(4-Aminophenyl)-4-bromo-3-*tert*-butoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (93 mg, 70%) was obtained as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.50 (s, 9 H), 3.79 (br, s, 2 H), 3.87 (s, 3 H), 4.82 (s, 2 H), 6.75 (dd, $J = 7.96$, 2.15 Hz, 1 H), 6.96 (t, $J = 2.02$ Hz, 1 H), 7.03 (d, $J = 7.58$ Hz, 1 H), 7.23 (t, $J = 7.83$ Hz, 1 H), 7.26 (s, 1 H). Anal. Calcd for $C_{18}H_{20}BrNO_5S$: C, 48.88; H, 4.56; N, 3.17. Found: C, 48.84; H, 4.45; N, 3.10.

4-Bromo-3-ethoxycarbonylmethoxy-5-(3-hydroxyphenyl)thiophene-2-carboxylic Acid Methyl Ester (6b). Compound **6b** (334 mg, 65%) was prepared by following the general procedure of Suzuki coupling, using 4,5-dibromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (500 mg, 1.24 mmol), $Pd(PPh_3)_4$ (143 mg, 0.12 mmol), KF (216 mg, 3.74 mmol) and 3-hydroxyphenylboronic acid (171 mg, 1.24 mmol) as starting

materials. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.32 (t, $J = 7.20$ Hz, 3 H), 3.85 (s, 3 H), 4.30 (q, $J = 7.07$ Hz, 2 H), 4.89 (s, 2 H), 6.04 (s, 1 H), 7.18 (m, 2 H), 7.30 (m, 1 H). MS [(+)ESI, *m/z*]: 437.42 $[M + Na]^+$.

4-Bromo-3-ethoxycarbonylmethoxy-5-(3-propionylaminophenyl)thiophene-2-carboxylic Acid Methyl Ester (7a). General Procedure for Acylation. To a 1.5 mL CH_2Cl_2 solution of 5-(3-aminophenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (44 mg, 0.1 mmol) were added diisopropylethyl amine (27 μL, 0.15 mmol) and propionyl chloride (11 μL, 0.12 mmol). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was then diluted with CH_2Cl_2; washed with dilute aqueous HCl, saturated $NaHCO_3$, and brine; and dried over $MgSO_4$. 4-Bromo-3-ethoxycarbonylmethoxy-5-(3-propionylaminophenyl)thiophene-2-carboxylic acid methyl ester (45 mg, 90%) was obtained as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.26 (t, $J = 7.58$ Hz, 3 H), 1.51 (s, 9 H), 2.43 (q, $J = 7.58$ Hz, 2 H), 3.87 (s, 3 H), 4.82 (s, 2 H), 7.38 (m, 2 H), 7.43 (s, 1 H), 7.63 (m, 1 H), 7.83 (s, 1 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-(3-methoxycarbonylaminophenyl)thiophene-2-carboxylic Acid Methyl Ester (7b). General Procedure for Carbamate Formation. To a 1.5 mL CH_2Cl_2 solution of 5-(3-amino-phenyl)-4-bromo-3-*tert*-butoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (42 mg, 0.095 mmol) was added diisopropylethylamine (34 μL, 0.19 mmol) followed by methyl chloroformate (13 μL, 0.17 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then diluted with CH_2Cl_2, washed with dilute aqueous HCl and saturated $NaHCO_3$, and dried over $MgSO_4$. Filtration and evaporation provided 4-bromo-3-*tert*-butoxycarbonylmethoxy-5-(3-methoxycarbonylaminophenyl)thiophene-2-carboxylic acid methyl ester (40 mg, 84%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.51 (s, 9 H), 3.80 (s, 3 H), 3.87 (s, 3 H), 4.82 (s, 2 H), 6.77 (s, 1 H), 7.35 (dt, $J = 7.64$, 1.61 Hz, 1 H), 7.39 (t, $J = 7.83$ Hz, 1 H), 7.47 (m, 1 H), 7.73 (s, 1 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(3-isopropylureido)phenyl]thiophene-2-carboxylic Acid Methyl Ester (7c). General Procedure for Urea Formation. To a 1 mL DMF solution of 5-(3-aminophenyl)-4-bromo-3-*tert*-butoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (35 mg, 0.079 mmol) was added isopropyl isocyanate (38 μL, 0.4 mmol), and the reaction mixture was stirred at 60 °C overnight. The reaction mixture was then cooled to room temperature, diluted with water, and extracted with EtOAc. The organic phase was washed with water and brine and dried over $MgSO_4$. The crude product was purified by flash column chromatography using a gradient of hexane/EtOAc (0−30% gradient) as eluent. Pure fractions were combined and evaporated to give 4-bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(3-isopropylureido)phenyl]thiophene-2-carboxylic acid methyl ester (21 mg, 50%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.08 (d, $J = 6.57$ Hz, 6 H), 1.44 (s, 9 H), 3.75 (s, 3 H), 3.93 (m, 1 H), 4.70 (s, 2 H), 5.13 (d, $J = 7.58$ Hz, 1 H), 7.14 (m, 2 H), 7.20 (t, $J = 7.83$ Hz, 1 H), 7.40 (m, 2 H).

4-Bromo-3-ethoxycarbonylmethoxy-5-[3-(4-fluorobenzenesulfonylamino)phenyl]thiophene-2-carboxylic Acid Methyl Ester (7d). General Procedure for Sulfonamide Formation. To a 1 mL pyridine solution of 5-(3-aminophenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (30 mg, 0.072 mmol) was added 4-fluorobenzenesulfonyl chloride (15 mg, 0.079 mmol), and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then diluted with 15 mL of 1 N HCl and extracted with EtOAc. The organic layer was washed with dilute aqueous HCl, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. Filtration and evaporation gave the crude product, which was purified by preparative TLC (35% EtOAc/hexane). The product band was isolated to give 4-bromo-3-ethoxycarbonylmethoxy-5-[3-(4-fluoro-benzenesulfonylamino)phenyl]-thiophene-2-carboxylic acid methyl ester (48 mg, 86%) as a colorless glassy solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.32 (t, $J = 7.20$ Hz, 3 H), 3.87 (s, 3 H), 4.30 (q, $J = 7.07$ Hz, 2 H), 4.90 (s, 2 H), 7.14 (m, 3 H), 7.20 (m, 1 H), 7.37 (m, 3 H), 7.85 (m, 2 H).

5-(3-Benzylaminophenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic Acid Methyl Ester (7e). General Procedure for Reductive Amination. To a 2 mL DCE solution of 5-(3-aminophenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (83 mg, 0.2 mmol) was added benzaldehyde (24 μL, 0.24 mmol) followed by acetic acid (20 μL, 0.3 mmol) and sodium triacetoxyborohydride (106 mg, 0.5 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was then diluted with CH_2Cl_2, washed with saturated $NaHCO_3$, and dried over $MgSO_4$. The crude product was purified by flash column chromatography using a gradient of EtOAc/hexane (5−25% gradient) as eluent. Pure fractions were combined and evaporated to give 5-(3-benzylaminophenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carbox ylic acid methyl ester (65 mg, 64%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.31 (t, $J = 7.07$ Hz, 3 H), 3.86 (s, 3 H), 4.24 (s, 1 H), 4.29 (q, $J = 7.16$ Hz, 2 H), 4.37 (s, 2 H), 4.88 (s, 2 H), 6.70 (m, 1 H), 6.92 (m, 1 H), 6.97 (dd, $J = 7.58$, 1.01 Hz, 1 H), 7.23 (t, $J = 7.83$ Hz, 1 H), 7.29 (m, 1 H), 7.37 (m, 4 H).

5-(3-Benzyloxyphenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic Acid Methyl Ester (7f). To a 4 mL solution of 4-bromo-3-ethoxycarbonylmethoxy-5-(3-hydroxyphenyl)thiophene-2-carboxylic acid methyl ester (50 mg, 0.12 mmol) in DMF were added benzyl bromide (22 μL, 0.18 mmol) and K_2CO_3 (33 mg, 0.24 mmol). The resulting suspension was stirred at 60 °C overnight. DMF was evaporated under reduced pressure. CH_2Cl_2 (20 mL) was then added, and the resulting organic solution was washed with water twice and brine once and then dried over anhydrous Na_2SO_4. The crude product was purified by flash column chromatography to give 5-(3-benzyloxyphenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (52 mg, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.32 (t, $J = 7.20$ Hz, 3 H), 3.87 (s, 3 H), 4.29 (q, $J = 7.24$ Hz, 2 H), 4.91 (s, 2 H), 5.12 (s, 2 H), 7.06 (m, 1 H), 7.24 (m, 1 H), 7.29 (m, 1 H), 7.39 (m, 6 H).

5-(3-Aminophenyl)-4-bromo-3-carboxymethoxythiophene-2-carboxylic Acid (8). General Procedure for Hydrolysis. To a 2 mL THF solution of 5-(3-amino-phenyl)-4-bromo-3-*tert*-butoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (**6a**; 16 mg, 0.036 mmol) was added 1 mL of 1 N LiOH solution. The mixture was stirred at room temperature overnight, and then THF was removed under reduced pressure. Aqueous HCl (1 N) was added slowly until the acidity of the solution reached pH ~1. The precipitate was filtered, washed with water, and dried to give 5-(3-aminophenyl)-4-bromo-3-carboxymethoxythiophene-2-carboxylic acid (9.5 mg, 71%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 4.88 (s, 2 H), 6.75 (d, $J = 7.07$ Hz, 1 H), 6.88 (d, $J = 7.07$ Hz, 1 H), 6.94 (s, 1 H), 7.20 (t, $J = 8.08$ Hz, 1 H).

4-Bromo-3-carboxymethoxy-5-(3-hydroxyphenyl)thiophene-2-carboxylic Acid (9). 4-Bromo-3-ethoxycarbonylmethoxy-5-(3-hydroxyphenyl)thiophene-2-carboxylic acid methyl ester (**6b**; 39 mg, 0.09 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-(3-hydroxyphenyl)-thiophene-2-carboxylic acid (26 mg, 73%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.88 (s, 2 H), 6.89 (m, 1 H), 7.07 (m, 2 H), 7.32 (t, $J = 8.08$ Hz, 1 H), 9.84 (s, 1 H). HRMS: calcd for $C_{13}H_9BrO_6S + H^+$, 372.937 60; found (ESI-FTMS, $[M + H]^+$), 372.937 85. RF-HPLC: H_2O/MeCN/0.1% formic acid, 99%; H_2O/MeCN/0.1% formic acid, 99%.

4-Bromo-3-carboxymethoxy-5-(3-methoxyphenyl)thiophene-2-carboxylic Acid (10). 4-Bromo-3-ethoxycarbonylmethoxy-5-(3-methoxyphenyl)thiophene-2-carboxylic acid methyl ester (38 mg, 59%) was synthesized according to the general procedure for Suzuki coupling, using 3-methoxyphenylboronic acid as the coupling partner in the reaction. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.33 (m, 3 H) 3.86 (s, 3 H) 3.88 (s, 3 H) 4.30 (q, $J = 7.16$ Hz, 2 H) 4.92 (s, 2 H) 6.98 (m, 1 H) 7.22 (m, 2 H) 7.37 (m, 1 H). MS [(+)-ESI, *m/z*]: 429.45 $[M + H]^+$.

4-Bromo-3-ethoxycarbonylmethoxy-5-(3-methoxyphenyl)thiophene-2-carboxylic acid methyl ester (38 mg, 0.088 mmol) was then hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-(3-methoxy-phenyl)-thiophene-2-carboxylic acid (25 mg, 71%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.82 (s, 3 H), 4.88 (s, 2 H), 7.08 (dd, J = 8.34, 2.27 Hz, 1 H), 7.22 (m, 2 H), 7.45 (t, J = 7.96 Hz, 1 H). HRMS: calcd for $C_{14}H_{11}BrO_6S + H^+$, 386.953 25; found (ESI-FTMS, $[M + H]^+$), 386.953 45. RF-HPLC: H_2O/MeCN/0.1% formic acid, 99%; H_2O/MeCN/0.1% formic acid, 99%.

4-Bromo-3-carboxymethoxy-5-(3-propionylaminophenyl)thiophene-2-carboxylic Acid (11). 4-Bromo-3-ethoxycarbonylmethoxy-5-(3-propionylaminophenyl)thiophene-2-carboxylic acid methyl ester (**7a**; 45 mg, 0.096 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-(3-propionylaminophenyl)thiophene-2-carboxylic acid (32 mg, 83%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.09 (t, J = 7.58 Hz, 3 H), 2.34 (m, 2 H), 4.89 (s, 2 H), 7.33 (s, 1 H), 7.44 (t, J = 7.83 Hz, 1 H), 7.67 (s, 1 H), 8.03 (s, 1 H), 10.08 (s, 1 H). HRMS: calcd for $C_{16}H_{14}BrNO_6S + H^+$, 427.979 80; found (ESI-FTMS, $[M + H]^+$), 427.980 16. RF-HPLC: H_2O/MeCN/0.1% formic acid, 98%; H_2O/MeCN/0.1% formic acid, 99%.

5-(3-Benzoylaminophenyl)-4-bromo-3-carboxymethoxythiophene-2-carboxylic Acid (12). 5-(3-Benzoylaminophenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (27 mg, 60%) was prepared as a colorless glassy solid according to the general procedure of acylation using 5-(3-aminophenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (36 mg, 0.087 mmol) and benzoyl chloride (14 μL, 0.12 mmol) as starting materials. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.32 (t, J = 7.07 Hz, 3 H), 3.88 (s, 3 H), 4.29 (q, J = 7.16 Hz, 2 H), 4.91 (s, 2 H), 7.48 (m, 4 H), 7.56 (m, 1 H), 7.76 (m, 1 H), 7.89 (m, 2 H), 7.98 (m, 2 H).

5-(3-Benzoylaminophenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (27 mg, 0.052 mmol) was then hydrolyzed according to the general procedure for hydrolysis to give 5-(3-benzoylaminophenyl)-4-bromo-3-carboxymethoxythiophene-2-carboxylic acid (27 mg, >95%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.90 (s, 2 H) 7.42 (m, 1 H) 7.53 (m, 3 H) 7.61 (m, 1 H) 7.91 (m, 1 H) 7.97 (m, 2 H) 8.21 (t, J = 1.77 Hz, 1 H) 10.46 (s, 1 H). HRMS: calcd for $C_{20}H_{14}BrNO_6S + H^+$, 475.979 80; found (ESI-FTMS, $[M + H]^+$), 475.980 43. RF-HPLC: H_2O/MeCN/0.1% formic acid, 95%; H_2O/MeCN/0.1% formic acid, 99%.

4-Bromo-3-carboxymethoxy-5-[3-(3-isopropylureido)phenyl]-thiophene-2-carboxylic Acid (13). 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-(3-methoxycarbonylaminophenyl)thiophene-2-carboxylic acid methyl ester (**7c**; 21 mg, 0.04 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-[3-(3-isopropylureido)phenyl]thiophene-2-carboxylic acid (14 mg, 77%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.10 (d, J = 6.57 Hz, 6 H), 3.75 (m, 1 H), 4.88 (s, 2 H), 6.07 (d, J = 7.83 Hz, 1 H), 7.18 (dd, J = 6.19, 1.64 Hz, 1 H), 7.35 (t, J = 7.83 Hz, 1 H), 7.42 (m, 1 H), 7.81 (t, J = 1.77 Hz, 1 H), 8.54 (s, 1 H). HRMS: calcd for $C_{17}H_{17}BrN_2O_6S + H^+$, 457.006 35; found (ESI-FTMS, $[M + H]^+$), 457.007 08. RF-HPLC: H_2O/MeCN/0.1% formic acid, 96%; H_2O/MeCN/0.1% formic acid, 96%.

4-Bromo-3-carboxymethoxy-5-(3-methoxycarbonylaminophenyl)thiophene-2-carboxylic Acid (14). 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-(3-methoxycarbonylaminophenyl)thiophene-2-carboxylic acid methyl ester (**7b**; 40 mg, 0.08 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-(3-methoxycarbonylaminophenyl)thiophene-2-carboxylic acid (9 mg, 26%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.69 (s, 3 H), 4.89 (s, 2 H), 7.29 (m, 1 H), 7.43 (t, J = 7.96 Hz, 1 H), 7.55 (d, J = 9.60 Hz, 1 H), 7.86 (s, 1 H), 9.90 (s, 1 H). HRMS: calcd for $C_{15}H_{12}BrNO_7S + H^+$, 429.959 06; found (ESI-FTMS, $[M + H]^+$), 429.959 73. RF-HPLC: H_2O/MeCN/0.1% formic acid, 96%; H_2O/MeCN/0.1% formic acid, 97%.

4-Bromo-3-carboxymethoxy-5-[3-(4-fluorobenzenesulfonylamino)phenyl]thiophene-2-carboxylic Acid (15). 4-Bromo-3-ethoxycarbonylmethoxy-5-[3-(4-fluorobenzenesulfonylamino)phenyl]-thiophene-2-carboxylic acid methyl ester (**7d**; 48 mg, 0.084 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-[3-(4-fluorobenzenesulfonylamino)phenyl]thiophene-2-carboxylic acid (43 mg, 96%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.89 (s, 2 H), 7.21 (m, 1 H), 7.32 (m, 1 H), 7.42 (m, 4 H), 7.86 (m, 2 H), 10.61 (s, 1 H). HRMS: calcd for $C_{19}H_{13}BrFNO_7S_2 + H^+$, 529.937 36; found (ESI-FTMS, $[M + H]^+$), 529.9384. RF-HPLC: H_2O/MeCN/0.1% formic acid, 96%; H_2O/MeCN/0.1% formic acid, 95%.

5-(3-Benzylaminophenyl)-4-bromo-3-carboxymethoxythiophene-2-carboxylic Acid (16). 5-(3-Benzylaminophenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (**7e**; 40 mg, 0.079 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 5-(3-benzylaminophenyl)-4-bromo-3-carboxymethoxythiophene-2-carboxylic acid (28 mg, 77%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.31 (s, 2 H), 4.85 (s, 2 H), 6.70 (d, J = 8.84 Hz, 1 H), 6.79 (d, J = 7.07 Hz, 1 H), 6.83 (s, 1 H), 7.20 (m, 2 H), 7.35 (m, 4 H). HRMS: calcd for $C_{20}H_{16}BrNO_5S + H^+$, 462.000 53; found (ESI-FTMS, $[M + H]^+$), 462.000 91. RF-HPLC: H_2O/MeCN/0.1% formic acid, 97%; H_2O/MeCN/0.1% formic acid, 97%.

5-(3-Benzyloxyphenyl)-4-bromo-3-carboxymethoxythiophene-2-carboxylic Acid (17). 5-(3-Benzyloxyphenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (**7f**; 52 mg, 0.10 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 5-(3-benzyloxyphenyl)-4-bromo-3-carboxymethoxythiophene-2-carboxylic acid (49 mg, >95%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.86 (s, 2 H), 5.18 (s, 2 H), 7.16 (dd, J = 7.96, 2.15 Hz, 1 H), 7.23 (dd, J = 6.44, 1.39 Hz, 1 H), 7.28 (m, 1 H), 7.35 (m, J = 7.33 Hz, 1 H), 7.44 (m, 5 H). RF-HPLC: H_2O/MeCN/0.1% formic acid, 100%; H_2O/MeCN/0.1% formic acid, 100%.

4-Bromo-3-carboxymethoxy-5-[3-(cyclohexylmethylamino)-phenyl]thiophene-2-carboxylic Acid (18). 4-Bromo-5-[3-(cyclohexylmethylamino)phenyl]-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was obtained in 69% yield as a pale yellow oil, by following the general procedure for reductive amination. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 0.99 (m, 2 H), 1.21 (m, 4 H), 1.32 (t, J = 7.20 Hz, 3 H), 1.70 (m, 5 H), 2.98 (d, J = 6.82 Hz, 2 H), 3.87 (s, 3 H), 4.30 (q, J = 7.24 Hz, 2 H), 4.90 (s, 2 H), 6.65 (m, 1 H), 6.85 (m, 1 H), 6.93 (d, J = 7.58 Hz, 1 H), 7.22 (t, J = 7.83 Hz, 1 H).

4-Bromo-5-[3-(cyclohexylmethylamino)phenyl]-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was then hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-[3-(cyclohexylmethylamino)-phenyl]thiophene-2-carboxylic acid in 59% yield as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.94 (m, 2 H), 1.20 (m, 4 H), 1.67 (m, 5 H), 2.87 (d, J = 6.57 Hz, 2 H), 4.87 (s, 2 H), 6.66 (dd, J = 8.21, 1.64 Hz, 1 H), 6.76 (d, J = 7.58 Hz, 1 H), 6.81 (m, 1 H), 7.17 (t, J = 7.96 Hz, 1 H). HRMS: calcd for $C_{20}H_{22}BrNO_5S + H^+$, 468.047 49; found (ESI-FTMS, $[M + H]^+$), 468.048 21. RF-HPLC: H_2O/MeCN/0.1% formic acid, 100%; H_2O/MeCN/0.1% formic acid, 100%.

4-Bromo-3-carboxymethoxy-5-(3-cyclohexylaminophenyl)thiophene-2-carboxylic Acid (19). 4-Bromo-5-(3-cyclohexylaminophenyl)-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (35 mg, 70%) was prepared as a yellow oil, according to the general procedure for reductive amination, from 5-(3-aminophenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (41 mg, 0.1 mmol) and cyclohexanone (13 μL, 0.12 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.21 (m, 3 H), 1.32 (m, 3 H), 1.39 (m, 2 H), 1.66 (m, 1 H), 1.77 (m, 2 H), 2.08 (dd, J = 12.76, 3.16 Hz, 2 H), 3.28 (m, 1 H), 3.70 (s, 1 H), 3.87 (s, 3 H), 4.30 (q, J = 7.07 Hz, 2 H), 4.90 (s, 2 H), 6.64 (dd, J = 7.71, 1.89 Hz, 1 H), 6.85 (m, 1 H), 6.90 (m, 1 H) 7.21 (t, J = 7.83 Hz, 1 H).

4-Bromo-5-(3-cyclohexylaminophenyl)-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (35 mg, 0.07 mmol) was then hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-(3-cyclohexylaminophenyl)-thiophene-2-carboxylic acid (26 mg, 83%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.18 (m, 5 H), 1.34 (m, 2 H), 1.60 (m, 1 H), 1.73 (m, 2 H), 1.95 (m, 1 H), 4.88 (s, 2 H), 6.70 (m, 1 H), 6.77 (m, 1 H), 6.84 (s, 1 H), 7.19 (t, J = 7.71 Hz, 1 H). HRMS: calcd for $C_{19}H_{20}BrNO_5S + H^+$, 454.031 83; found (ESI-FTMS, $[M + H]^+$), 454.0323. RF-HPLC: H_2O/MeCN/0.1% formic acid, 100%; H_2O/MeCN/0.1% formic acid, 100%.

4-Bromo-3-carboxymethoxy-5-(3-cyclopentylaminophenyl)thiophene-2-carboxylic Acid (20). 4-Bromo-5-(3-cyclopentylaminophenyl)-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was obtained in 75% yield as a yellow glassy solid, by following the general procedure for reductive amination. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.32 (t, J = 7.07 Hz, 3 H), 1.50 (m, 2 H), 1.69 (m, 4 H), 2.05 (m, 2 H), 3.81 (m, 1 H), 3.87 (s, 3 H), 4.30 (q, J = 7.16 Hz, 2 H), 4.90 (s, 2 H), 6.65 (m, 1 H), 6.87 (m, 1 H), 6.93 (m, 1 H), 7.22 (t, J = 7.96 Hz, 1 H).

4-Bromo-5-(3-cyclopentylaminophenyl)-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was then hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-(3-cyclopentylaminophenyl)thiophene-2-carboxylic acid in 79% yield as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.47 (m, 2 H), 1.56 (m, 2 H), 1.67 (m, 2 H), 1.93 (m, 2 H), 3.71 (m, 1 H), 4.88 (s, 2 H), 6.67 (m, 1 H), 6.77 (d, J = 1.26 Hz, 1 H), 6.82 (d, J = 2.02 Hz, 1 H), 7.17 (m, 1 H). HRMS: calcd for $C_{18}H_{18}BrNO_5S + H^+$, 440.016 19; found (ESI-FTMS, $[M + H]^+$), 440.017 24. RF-HPLC: H_2O/MeCN/0.1% formic acid, 100%; H_2O/MeCN/0.1% formic acid, 100%.

4-Bromo-3-carboxymethoxy-5-(3-cycloheptylaminophenyl)thiophene-2-carboxylic Acid (21). 4-Bromo-5-(3-cycloheptylaminophenyl)-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was obtained in 72% yield as a pale yellow glassy solid, by following the general procedure for reductive amination. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.32 (t, J = 7.07 Hz, 3 H), 1.57 (m, 10 H), 2.03 (m, 2 H), 3.47 (m, 1 H), 3.76 (s, 1 H), 3.87 (s, 3 H), 4.30 (q, J = 7.16 Hz, 2 H), 4.90 (s, 2 H), 6.60 (dd, J = 8.21, 2.40 Hz, 1 H), 6.81 (t, J = 1.89 Hz, 1 H), 6.90 (d, J = 7.58 Hz, 1 H), 7.21 (t, J = 7.96 Hz, 1 H).

4-Bromo-5-(3-cycloheptylaminophenyl)-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was then hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-(3-cycloheptylaminophenyl)thiophene-2-carboxylic acid in 71% yield as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.53 (m, 10 H), 1.90 (t, J = 6.82 Hz, 2 H), 4.88 (s, 2 H), 6.65 (dd, J = 8.59, 2.27 Hz, 1 H), 6.77 (m, 2 H), 7.19 (t, J = 7.83 Hz, 1 H). HRMS: calcd for $C_{20}H_{22}BrNO_5S + H^+$, 468.047 48; found (ESI-FTMS, $[M + H]^+$), 468.048 17. RF-HPLC: H_2O/MeCN/0.1% formic acid, 96%; H_2O/MeCN/0.1% formic acid, 98%.

4-Bromo-3-carboxymethoxy-5-[3-(tetrahydropyran-4-ylamino)phenyl]thiophene-2-carboxylic Acid (22). 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(tetrahydropyran-4-ylamino)phenyl]-thiophene-2-carboxylic acid methyl ester was obtained in 83% yield as a pale yellow glassy solid, by following the general procedure for reductive amination. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.51 (m, 13 H), 2.06 (m, 2 H), 3.53 (m, 3 H), 3.72 (s, 1 H), 3.87 (s, 3 H), 4.82 (s, 2 H), 6.66 (dd, J = 7.83, 2.02 Hz, 1 H), 6.88 (m, 1 H), 6.95 (m, 1 H), 7.23 (t, J = 7.96 Hz, 1 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(tetrahydropyran-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester was then hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-[3-(tetrahydropyran-4-ylamino)phenyl]thiophene-2-carboxylic acid in 84% yield as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.39 (m, 2 H), 1.90 (d, J = 11.37 Hz, 2 H), 3.44 (m, 4 H), 3.86 (m, 2 H), 4.87 (s, 2 H), 6.72 (dd, J = 8.34, 2.27 Hz, 1 H), 6.77 (d, J = 7.58 Hz, 1 H), 6.85 (s, 1 H), 7.19 (t, J = 7.96 Hz, 1 H). HRMS: calcd for $C_{18}H_{18}BrNO_6S + H^+$, 456.011 10; found (ESI-FTMS, $[M + H]^+$), 456.011 53. RF-HPLC: H_2O/MeCN/0.1% formic acid, 98%; H_2O/MeCN/0.1% formic acid, 99%.

4-Bromo-3-carboxymethoxy-5-[3-(3,3,5,5-tetramethylcyclohexylamino)phenyl]thiophene-2-carboxylic Acid (23). 4-Bromo-3-ethoxycarbonylmethoxy-5-[3-(3,3,5,5-tetramethylcyclohexylamino)-phenyl]thiophene-2-carboxylic acid methyl ester was obtained in 56% yield as a pale yellow glassy solid, by following the general procedure for reductive amination. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 0.90 (m, 2 H), 0.94 (s, 6 H), 1.05 (m, 2 H), 1.12 (s, 6 H), 1.29 (s, 1 H), 1.32 (t, J = 7.20 Hz, 3 H), 1.89 (d, J = 11.87 Hz, 1 H), 3.61 (m, 2 H), 3.87 (s, 3 H), 4.30 (q, J = 7.16 Hz, 2 H), 4.89 (s, 2 H), 6.63 (dd, J = 7.07, 1.26 Hz, 1 H), 6.90 (m, 2 H), 7.21 (t, J = 7.96 Hz, 1 H).

4-Bromo-3-ethoxycarbonylmethoxy-5-[3-(3,3,5,5-tetramethylcyclohexylamino)phenyl]thiophene-2-carboxylic acid methyl ester was then hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-[3-(3,3,5,5-tetramethylcyclohexylamino)phenyl]thiophene-2-carboxylic acid in 66% yield as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.91 (s, 6 H), 0.95 (m, 2 H), 1.09 (m, 7 H), 1.26 (m, 1 H), 1.75 (d, J = 12.38 Hz, 2 H), 3.51 (m, 1 H), 4.87 (s, 2 H), 6.68 (dd, J = 8.21, 1.64 Hz, 1 H), 6.76 (d, J = 7.33 Hz, 1 H), 6.84 (s, 1 H), 7.18 (t, J = 7.96 Hz, 1 H). Anal. Calcd for $C_{23}H_{28}BrNO_5S$: C, 54.12; H, 5.53; N, 2.74. Found: C, 54.41; H, 5.21; N, 2.60. RF-HPLC: H_2O/MeCN/0.1% formic acid, 100%; H_2O/MeCN/0.1% formic acid, 100%.

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(piperidin-4-ylamino)phenyl]thiophene-2-carboxylic Acid Methyl Ester Hydrochloride (24). To a 12 mL DCE solution of 5-(3-amino-phenyl)-4-bromo-3-*tert*-butoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (1.33 g, 3.0 mmol) were added *tert*-butyl-4-oxo-1-piperidinecarboxylate (0.897 g, 4.5 mmol), acetic acid (0.25 mL, 4.5 mmol), and sodium triacetoxyborohydride (1.48 g, 7.0 mmol), and the resulting mixture was stirred at room temperature overnight. The reaction mixture was then diluted with CH_2Cl_2, washed twice with saturated $NaHCO_3$, and dried over $MgSO_4$. Filtration and evaporation gave the crude product, which was purified by flash column chromatography using a gradient of hexane/EtOAc (5−35% gradient) as eluent. Pure fractions were combined and evaporated to give 4-[3-(3-bromo-4-*tert*-butoxycarbonylmethoxy-5-methoxycarbonylthiophen-2-yl)phenylamino]piperidine-1-carboxylic acid *tert*-butyl ester (1.62 g, 86%) as a pale yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.37 (m, 2 H), 1.47 (s, 9 H), 1.51 (s, 9 H), 2.07 (m, 2 H), 2.94 (t, J = 12.13 Hz, 2 H), 3.47 (m, 1 H), 3.69 (m, 1 H), 3.87 (s, 3 H), 4.06 (m, 2 H), 4.82 (s, 2 H), 6.65 (m, 1 H), 6.87 (m, 1 H), 6.94 (ddd, J = 7.64, 1.58, 0.88 Hz, 1 H), 7.23 (t, J = 7.96 Hz, 1 H).

4-[3-(3-Bromo-4-*tert*-butoxycarbonylmethoxy-5-methoxycarbonylthiophen-2-yl)phenylamino]piperidine-1-carboxylic acid *tert*-butyl ester (1.33 g, 2.1 mmol) was dissolved in 10.5 mL of 1 N HCl in EtOAc (prepared by bubbling dry HCl gas into dry EtOAc) and 2 mL of MeOH. The reaction mixture was stirred at room temperature overnight. The reaction mixture was then evaporated exhaustively to give 4-bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(piperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester hydrochloride in quantitative yield as a white solid. ^{1}H NMR (400 MHz, MeOD): δ ppm 1.46 (s, 9 H), 2.00 (m, 2 H), 2.26 (m, 2 H), 3.09 (td, J = 12.95, 2.65 Hz, 1 H), 3.51 (m, 2 H), 3.84 (s, 3 H), 3.89 (m, 2 H), 4.82 (s, 2 H), 7.47 (m, 1 H), 7.61 (m, 2 H), 7.67 (m, 1 H).

4-Bromo-3-ethoxycarbonylmethoxy-5-[3-(piperidin-4-ylamino)-phenyl]thiophene-2-carboxylic acid methyl ester hydrochloride salt was prepared by following the same procedure, except that 5-(3-aminophenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was used as starting material in the reductive amination step. ^{1}H NMR (400 MHz, CD_3OD): δ ppm 1.48 (t, J = 7.20 Hz, 3 H), 1.81−1.94 (m, 2 H), 2.41−2.49 (m, 2 H), 3.30−3.38 (m, 2 H), 3.59−3.66 (m, 2 H), 3.82−3.89 (m, 1 H), 4.03 (s, 3 H), 4.44 (q, J = 7.07 Hz, 2 H), 5.08 (s, 2 H), 6.94−6.98 (m, 1 H), 7.06−7.09 (m, 1 H), 7.11−7.13 (m, 1 H), 7.39−7.44 (m, 1 H).

4-[3-(3-Bromo-4-ethoxycarbonylmethoxy-5-methoxycarbonylthiophen-2-yl)phenylamino]piperidine-1-carboxylic Acid Methyl Ester (25a). General Procedure for Carbamate Formation at the Piperidine Nitrogen. To a 1 mL pyridine solution of 4-bromo-3-ethoxycarbonylmethoxy-5-[3-(piperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester hydrochloride salt (60 mg, 0.1 mmol) was added methyl chloroformate (18 μL, 0.24 mmol). The reaction mixture was stirred at room temperature for 5 h, and then 10 mL of 2 N HCl was added and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. Filtration and evaporation gave the crude product, which was purified by flash column chromatography using a gradient of EtOAc/hexane (20−60% gradient) as eluent. Pure fractions were combined and evaporated to give 4-[3-(3-bromo-4-ethoxycarbonylmethoxy-5-methoxycarbonylthiophen-2-yl)phenylamino]piperidine-1-carboxylic acid methyl ester (15 mg, 27%) as a pale glassy solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.32 (t, J = 7.07 Hz, 3 H), 1.41 (m, 2 H), 2.09 (m, 2 H), 3.02 (m, 2 H), 3.48 (m, 1 H), 3.71 (s, 3 H), 3.87 (s, 3 H), 4.11 (m, 2 H), 4.30 (q, J = 7.07 Hz, 2 H), 4.90 (s, 2 H), 6.66 (m, 1 H), 6.87 (m, 1 H), 6.95 (m, 1 H), 7.23 (t, J = 7.83 Hz, 1 H).

5-[3-(1-Benzylcarbamoylpiperidin-4-ylamino)phenyl]-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic Acid Methyl Ester (25b). General Procedure for Urea Formation at the Piperidine Nitrogen. To a 1 mL DMF solution of 4-bromo-3-ethoxycarbonylmethoxy-5-[3-(piperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester (50 mg, 0.1 mmol) was added benzyl isocyanate (14 μL, 0.11 mmol) and the reaction mixture was stirred at room temperature. After 3 h, the reaction mixture was diluted with water, acidified with 1 N HCl, and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. Filtration and evaporation gave the crude product, which was then purified by flash column chromatography using a gradient of EtOAc/hexane (20−100% gradient) as eluent. Pure fractions were combined and evaporated to give 5-[3-(1-benzylcarbamoylpiperidin-4-ylamino)phenyl]-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (18 mg, 29%) as a pale yellow glassy solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.32 (t, J = 7.07 Hz, 3 H), 1.42 (dd, J = 13.64, 2.27 Hz, 2 H), 2.10 (m, 2 H), 3.01 (m, 2 H), 3.49 (m, 1 H), 3.74 (s, 1 H), 3.87 (s, 3 H), 3.94 (m, 2 H), 4.29 (q, J = 7.07 Hz, 2 H), 4.43 (d, J = 5.31 Hz, 2 H), 4.80 (t, J = 5.31 Hz, 1 H), 4.90 (s, 2 H), 6.65 (m, 1 H), 6.87 (m, 1 H), 6.95 (d, J = 8.34 Hz, 1 H), 7.23 (t, J = 7.96 Hz, 1 H), 7.31 (m, 5 H).

4-Bromo-3-ethoxycarbonylmethoxy-5-[3-(1-methanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic Acid Methyl Ester (25c). General Procedure for Sulfonamide Formation at the Piperidine Nitrogen. To a 1 mL pyridine solution of 4-bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(piperidin-4-ylamino)-phenyl]thiophene-2-carboxylic acid methyl ester hydrochloride (50 mg, 0.1 mmol) was added methanesulfonyl chloride (9 μL, 0.12 mmol), and the reaction mixture was stirred at room temperature overnight. The reaction was quenched with 8 mL of 2 N HCl and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. Filtration and evaporation gave the crude product, which was purified by flash column chromatography using a gradient of hexane/EtOAc (20−60% gradient) as eluent. Pure fractions were combined and evaporated to give 4-bromo-3-ethoxycarbonylmethoxy-5-[3-(1-methanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester (12 mg, 21%) as a pale yellow glassy solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.32 (t, J = 7.20 Hz, 3 H), 1.59 (m, 2 H), 2.20 (m, 2 H), 2.82 (s, 3 H), 2.92 (m, 2 H), 3.46 (dd, J = 11.87, 8.34 Hz, 1 H), 3.78 (m, 2 H), 3.88 (s, 3 H), 4.30 (q, J = 7.16 Hz, 2 H), 4.90 (s, 2 H), 6.66 (ddd, J = 8.15, 2.46, 0.76 Hz, 1 H), 6.87 (m, 1 H), 6.97 (ddd, J = 7.58, 1.64, 0.88 Hz, 1 H), 7.24 (t, J = 7.96 Hz, 1 H).

4-Bromo-3-(carboxymethoxy)-5-[3-(piperidin-4-ylamino)phenyl]thiophene-2-carboxylic Acid (26). 4-Bromo-3-ethoxycarbonylmethoxy-5-[3-(piperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester HCl salt was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-(carboxymethoxy)-5-[3-(piperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid (46 mg, 49%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.58 (m, 2 H), 2.05 (m, 2 H), 3.03 (m, 2 H), 3.38 (m, 2 H), 3.57 (m, 1 H), 4.77 (s, 2 H), 6.03 (d, J = 7.58 Hz, 1 H), 6.72 (dd, J = 8.08, 1.77 Hz, 1 H), 6.80 (d, J = 7.58 Hz, 1 H), 6.84 (s, 1 H), 7.20 (t, J = 7.96 Hz, 1 H), 8.45 (s, 1 H), 8.60 (s, 1 H). RF-HPLC: H_2O/MeCN/0.1% formic acid, 100%; H_2O/MeCN/0.1% formic acid, 100%.

5-[3-(1-Acetylpiperidin-4-ylamino)phenyl]-4-bromo-3-carboxymethoxythiophene-2-carboxylic Acid (27). 5-[3-(1-Acetylpiperidin-4-ylamino)phenyl]-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was obtained in 66% yield as a yellow oil, by following the general procedure for reductive amination except that 1-acetylpiperidin-4-one was used as starting material in the reaction. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.32 (t, J = 7.20 Hz, 2 H), 1.40 (m, 2 H), 2.17 (m, 6 H), 2.49 (dd, J = 6.57, 2.27 Hz, 1 H), 2.88 (m, 1 H), 3.23 (m, 1 H), 3.55 (s, 1 H), 3.74 (m, 1 H), 3.83 (m, 1 H), 3.88 (s, 3 H), 4.30 (q, J = 7.16 Hz, 1 H), 4.50 (m, 1 H), 4.91 (s, 2 H), 6.67 (m, 1 H), 6.88 (m, 1 H), 6.96 (m, 1 H), 7.24 (t, J = 7.83 Hz, 1 H).

5-[3-(1-Acetylpiperidin-4-ylamino)phenyl]-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was then hydrolyzed according to the general procedure for hydrolysis to give 5-[3-(1-acetylpiperidin-4-ylamino)phenyl]-4-bromo-3-carboxymethoxythiophene-2-carboxylic acid in 51% yield as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.25 (m, 2 H), 1.94 (m, 2 H), 2.00 (s, 3 H), 2.82 (m, 1 H), 3.18 (m, 2 H), 3.52 (s, 1 H), 3.78 (m, 1 H), 4.21 (m, 1 H), 4.84 (s, 2 H), 6.72 (m, 1 H), 6.77 (d, J = 7.58 Hz, 1 H), 6.85 (s, 1 H), 7.19 (t, J = 7.83 Hz, 1 H). HRMS: calcd for $C_{20}H_{21}BrN_2O_6S + H^+$, 497.037 65; found (ESI-FTMS, $[M + H]^+$), 497.038 93. RF-HPLC: H_2O/MeCN/0.1% formic acid, 96%; H_2O/MeCN/0.1% formic acid, 98%.

4-Bromo-3-(carboxymethoxy)-5-(3-(1-(methoxycarbonyl)piperidin-4-ylamino)phenyl)thiophene-2-carboxylic Acid (28). Compound **25a** (15 mg, 0.027 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-(carboxymethoxy)-5-(3-(1-(methoxycarbonyl)piperidin-4-ylamino)phenyl)thiophene-2-carboxylic acid (10 mg, 72%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.27 (m, 2 H), 1.92 (m, 2 H), 3.01 (m, 2 H), 3.46 (m, 1 H), 3.59 (s, 3 H), 3.91 (m, 2 H), 4.87 (s, 2 H), 6.71 (dd, J = 8.21, 2.40 Hz, 1 H), 6.78 (d, J = 7.58 Hz, 1 H), 6.85 (s, 1 H), 7.19 (t, J = 7.83 Hz, 1 H). HRMS: calcd for $C_{20}H_{21}BrN_2O_7S + H^+$, 513.032 56; found (ESI-FTMS, $[M + H]^+$), 513.033 14. RF-HPLC: H_2O/MeCN/0.1% formic acid, 97%; H_2O/MeCN/0.1% formic acid, 98%.

5-[3-(1-Benzylcarbamoylpiperidin-4-ylamino)phenyl]-4-bromo-3-carboxymethoxythiophene-2-carboxylic Acid (29). Compound **25b** (18 mg, 0.029 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 5-[3-(1-benzylcarbamoylpiperidin-4-ylamino)phenyl]-4-bromo-3-carboxymethoxythiophene-2-carboxylic acid (16 mg, 94%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.29 (m, 2 H), 1.90 (m, 2 H), 2.91 (m, 2 H), 3.47 (m, 1 H), 3.93 (dd, J = 12.63, 1.52 Hz, 2 H), 4.24 (d, J = 5.81 Hz, 2 H), 4.88 (s, 2 H), 6.72 (dd, J = 8.21, 2.15 Hz, 1 H), 6.78 (d, J = 8.59 Hz, 1 H), 6.85 (m, 1 H), 7.08 (t, J = 6.32 Hz, 1 H), 7.24 (m, 5 H). HRMS: calcd for $C_{26}H_{26}BrN_3O_6S + H^+$, 588.079 84; found (ESI-FTMS, $[M + H]^+$), 588.080 57. RF-HPLC: H_2O/MeCN/0.1% formic acid, 95%; H_2O/MeCN/0.1% formic acid, 95%.

4-Bromo-3-carboxymethoxy-5-[3-(1-methanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic Acid (30). Compound **25c** (12 mg, 0.021 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-[3-(1-methanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid (6 mg, 54%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.53 (d, J = 11.37 Hz, 2 H), 4.87 (s, 2 H), 6.72 (dd, J = 8.21, 1.64 Hz, 1 H), 6.79 (d, J = 8.08 Hz, 1 H), 6.85 (s, 1 H), 7.20 (t, J = 8.08 Hz, 1 H). HRMS: calcd for $C_{19}H_{21}BrN_2O_7S_2 + H^+$, 533.004 64; found (ESI-FTMS, $[M + H]^+$),

533.005 55. RF-HPLC: H_2O/MeCN/0.1% formic acid, 100%; H_2O/MeCN/0.1% formic acid, 100%.

5-[3-(1-Benzenesulfonylpiperidin-4-ylamino)phenyl]-4-bromo-3-carboxymethoxythiophene-2-carboxylic Acid (31). 5-[3-(1-Benzenesulfonylpiperidin-4-ylamino)phenyl]-4-bromo-3-*tert*-butoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was obtained as a colorless glassy solid in 55% yield by following the general procedure for sulfonamide formation at the piperidine nitrogen, except that phenylsulfonyl chloride was used as starting material. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.50 (s, 9 H), 1.56 (m, 2 H), 2.14 (m, 2 H), 2.54 (td, J = 11.62, 2.53 Hz, 2 H), 3.28 (m, 1 H), 3.65 (s, 1 H), 3.74 (m, 2 H), 3.86 (s, 3 H), 4.80 (s, 2 H), 6.59 (dd, J = 7.83, 2.02 Hz, 1 H), 6.78 (m, 1 H), 6.93 (d, J = 7.58 Hz, 1 H), 7.19 (t, J = 7.83 Hz, 1 H), 7.60 (m, 3 H), 7.78 (m, 2 H).

5-[3-(1-Benzenesulfonylpiperidin-4-ylamino)phenyl]-4-bromo-3-*tert*-butoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was then hydrolyzed according to the general procedure for hydrolysis to give 5-[3-(1-benzenesulfonylpiperidin-4-ylamino)-phenyl]-4-bromo-3-carboxymethoxythiophene-2-carboxylic acid in 82% yield as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.44 (m, 2 H), 1.98 (m, 2 H), 3.56 (d, J = 11.37 Hz, 2 H), 4.85 (s, 2 H), 6.66 (dd, J = 7.71, 1.89 Hz, 1 H), 6.76 (m, 2 H), 7.15 (t, J = 7.71 Hz, 1 H), 7.66 (t, J = 7.07 Hz, 2 H), 7.75 (m, 3 H). HRMS: calcd for $C_{24}H_{23}BrN_2O_7S_2 + H^+$, 595.020 28; found (ESI-FTMS, $[M + H]^+$), 595.020 88. RF-HPLC: H_2O/MeCN/0.1% formic acid, 95%; H_2O/MeCN/0.1% formic acid, 97%.

4-Bromo-3-carboxymethoxy-5-[3-(1-phenylmethanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic Acid (32). 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(piperidin-4-ylamino)phenyl]-thiophene-2-carboxylic acid methyl ester hydrochloride (56 mg, 0.1 mmol) was dissolved in a vigorously stirred mixture of 0.5 mL of CH_2Cl_2 and 0.5 mL of saturated sodium bicarbonate solution. α-Toluenesulfonyl chloride (23 mg, 0.12 mmol) was then added and the biphasic mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with CH_2Cl_2, washed with saturated sodium bicarbonate solution, and dried over $MgSO_4$. Filtration and evaporation gave the crude product, which was purified by flash column chromatography using a gradient of EtOAc/hexane (5−35% gradient) as eluent. Pure fractions were combined and evaporated to give 4-bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(1-phenylmethanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester (47 mg, 69%) as a colorless glassy solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.41 (m, 2 H), 1.51 (s, 9 H), 2.03 (m, 2 H), 2.78 (m, 2 H), 3.35 (m, 1 H), 3.62 (m, 2 H), 3.87 (s, 3 H), 4.24 (s, 2 H), 4.82 (s, 2 H), 6.61 (dd, J = 7.83, 2.02 Hz, 1 H), 6.83 (m, 1 H), 6.94 (d, J = 8.08 Hz, 1 H), 7.21 (t, J = 7.83 Hz, 1 H), 7.40 (m, 5 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(1-phenylmethanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester (47 mg, 0.069 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-[3-(1-phenylmethanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid (37 mg, 88%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.36 (m, 2 H), 1.96 (m, 2 H), 2.92 (m, 2 H), 3.53 (m, 2 H), 4.40 (s, 2 H), 4.87 (s, 2 H), 6.71 (dd, J = 8.08, 1.52 Hz, 1 H), 6.79 (d, J = 7.58 Hz, 1 H), 6.84 (t, J = 1.89 Hz, 1 H), 7.20 (t, J = 7.83 Hz, 1 H), 7.40 (m, 5 H). Anal. Calcd for $C_{25}H_{25}BrN_2O_7S_2 \cdot H_2O$: C, 47.85; H, 4.34; N, 4.46. Found: C, 48.16; H, 4.43; N, 4.32. RF-HPLC: H_2O/MeCN/0.1% formic acid, 99%; H_2O/MeCN/0.1% formic acid, 98%.

5-(3-{[1-(Benzylsulfonyl)piperidin-4-yl]amino}phenyl)-3-(carboxymethoxy)thiophene-2-carboxylic Acid (33). To a round-bottomed flask were added 4-bromo-3-carboxymethoxy-5-[3-(1-phenylmethanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester (160 mg, 0.26 mmol) and ethanol (20 mL). The solution was cooled in a water bath and then excess sodium borohydride was added. The mixture was stirred for overnight at room temperature and water (20 mL) was then added. The pH of the solution was adjusted to ∼4 with 1 N aqueous HCl. The reaction mixture was extracted with ethyl acetate (20 mL × 3). The organic layers were combined and washed with brine (10 mL × 2). The crude product was purified by preparative HPLC to give two products, {4-bromo-2-hydroxymethyl-5-[3-(1-phenylmethanesulfonylpiperidin-4-ylamino)phenyl]thiophen-3-yloxy}-acetic acid (100 mg, 64%) and 3-carboxymethoxy-5-[3-(1-phenylmethanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester (30 mg, 21%).

3-Carboxymethoxy-5-[3-(1-phenylmethanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester (30 mg, 0.055 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 3-carboxymethoxy-5-[3-(1-phenylmethanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid (16 mg, 55%) as an off-white solid after preparative HPLC purification. ^{1}H NMR (400 MHz, MeOD): δ ppm 1.55 (m, 2 H), 2.03 (m, 2 H), 2.84 (m, 2 H), 3.57 (m, 1 H), 3.69 (d, J = 12.63 Hz, 2 H), 4.35 (s, 2 H), 4.89 (s, 2 H), 7.01 (d, J = 7.33 Hz, 1 H), 7.26 (s, 2 H), 7.40 (m, 7 H). HRMS: calcd for $C_{25}H_{26}N_2O_7S_2 + H^+$, 531.125 42; found (ESI-FTMS, $[M + H]^+$), 531.125 45. RF-HPLC: H_2O/MeCN/0.1% formic acid, 100%; H_2O/MeCN/0.1% formic acid, 100%.

5-(3-{[1-(Benzylsulfonyl)piperidin-4-yl]amino}phenyl)-3-(carboxymethoxy)-4-methylthiophene-2-carboxylic Acid (34). A round-bottom flask equipped with a distillation head was charged with 3-hydroxy-4-methylthiophene-2-carboxylic acid methyl ester[39] (1.5 g, 8.7 mmol) and acetic acid (22 mL). The stirred reaction was treated with Br_2 (540 μL, 11 mmol) and then heated to 70 °C. After 18 h of heating, the reaction was cooled to room temperature and the acetic acid was removed under reduced pressure to provide 5-bromo-3-hydroxy-4-methylthiophene-2-carboxylic acid methyl ester (2.19 g, 99%) as a light red solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 2.08 (s, 3 H), 3.88 (s, 3 H).

5-Bromo-3-ethoxycarbonylmethoxy-4-methylthiophene-2-carboxylic acid methyl ester was prepared from methyl 5-bromo-3-hydroxy-4-methylthiophene-2-carboxylate by following the general procedure for alkylation to give an orange solid (2.94 g, 74%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.29 (t, J = 7.20 Hz, 3 H), 2.16 (m, 3 H), 3.83 (m, 3 H), 4.24 (q, J = 7.16 Hz, 2 H), 4.89 (s, 2 H).

5-(3-Aminophenyl)-3-ethoxycarbonylmethoxy-4-methylthiophene-2-carboxylic acid methyl ester was prepared from 5-bromo-3-ethoxycarbonylmethoxy-4-methylthiophene-2-carboxylic acid methyl ester by following the general procedure for Suzuki coupling to give a white solid (350 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.30 (t, J = 7.07 Hz, 3 H), 2.26 (s, 3 H), 3.76 (s, 2 H), 3.85 (s, 3 H), 4.26 (q, J = 7.07 Hz, 2 H), 4.91 (s, 2 H), 6.70 (m, 1 H), 6.76 (m, J = 1.89, 1.89 Hz, 1 H), 6.85 (m, 1 H), 7.21 (t, J = 7.83 Hz, 1 H).

tert-Butyl 4-(3-(4-(2-*tert*-butoxy-2-oxoethoxy)-5-(methoxycarbonyl)-3-methylthiophen-2-yl)phenylamino)piperidine-1-carboxylate was prepared by following the general procedure for reductive amination to give a yellow solid (2.17 g, 97%).

tert-Butyl-4-(3-(4-(2-*tert*-butoxy-2-oxoethoxy)-5-(methoxycarbonyl)-3-methylthiophen-2-yl)phenylamino)piperidine-1-carboxylate was dissolved in 1 N HCl in EtOAc and MeOH. The reaction was stirred at room temperature for overnight. The solvent was removed under reduced pressure to give methyl-3-(2-*tert*-butoxy-2-oxoethoxy)-4-methyl-5-(3-(piperidin-4-ylamino)phenyl)thiophene-2-carboxylate hydrochloride as a white solid (1.5 g, 83%).

Methyl-5-(3-(1-(benzylsulfonyl)piperidin-4-ylamino)phenyl)-3-(2-*tert*-butoxy-2-oxoethoxy)-4-methylthiophene-2-carboxylate was prepared by following the general procedure for sulfonamide formation at the piperidine nitrogen.

Methyl-5-(3-(1-(benzylsulfonyl)piperidin-4-ylamino)phenyl)-3-(2-*tert*-butoxy-2-oxoethoxy)-4-methylthiophene-2-carboxylate was hydrolyzed by following the general procedure for hydrolysis to give compound **34** (96 mg, 71%) a pale beige solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.36 (m, 2 H), 1.91 (m, 2 H), 2.18 (s, 3 H), 2.92 (s, 2 H), 3.42 (m, 1 H), 3.56 (m, 2 H), 4.40 (s, 2 H), 4.86 (s, 2 H), 6.69 (s, 2 H), 7.18 (m, 1 H), 7.40 (m, 6 H). HRMS: calcd for $C_{26}H_{28}N_2O_7S_2 + H^+$, 545.141 07; found (ESI-FTMS, $[M + H]^+$), 545.1423. RF-HPLC: H_2O/MeCN/0.1% formic acid, 96%; H_2O/MeCN/0.1% formic acid, 100%.

5-(3-{[1-(Benzylsulfonyl)piperidin-4-yl]amino}phenyl)-3-(carboxymethoxy)-4-chlorothiophene-2-carboxylic Acid (35). Methyl 4,5-dichloro-3-hydroxythiophene-2-carboxylate was prepared according to a published procedure.[33] A solution of methyl 4,5-dichloro-3-hydroxythiophene-2-carboxylate (6.3 g, 20 mmol), *tert*-butyl bromoacetate (4.9 mL, 24 mmol), and K_2CO_3 (4.6 g, 24 mmol) in DMF (50 mL) was heated at 60 °C for 1 h. The cooled solution was diluted with ethyl acetate (400 mL), extracted with water (4 × 100 mL), washed with brine solution, dried over magnesium sulfate, filtered, and evaporated. The crude product was purified by flash column chromatography (20% EtOAc/hexane as eluent) to provide methyl 3-(2-*tert*-butoxy-2-oxoethoxy)-4,5-dichlorothiophene-2-carboxylate (8.4 g, 88%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.48 (s, 9 H), 3.85 (s, 3 H), 4.84 (s, 2 H).

A solution of methyl 3-(2-*tert*-butoxy-2-oxoethoxy)-4,5-dichlorothiophene-2-carboxylate (3.0 g, 8.8 mmol), 3-aminophenylboronic acid (1.29 g, 10.5 mmol), $Pd_2(dba)_3$ (400 mg, 0.44 mmol), KF (1.5 g, 26.4 mmol), and tri-*tert*-butylphosphine tetrafluoroborate (255 mg, 0.88 mmol) in dioxane (30 mL) was heated in a sealed tube at 70 °C for 48 h. The cooled solution was absorbed onto silica gel and solvent was evaporated. The crude product was purified by flash column chromatography (20−30% EtOAc/hexane as eluent) to provide methyl 5-(3-aminophenyl)-3-(2-*tert*-butoxy-2-oxoethoxy)-4-chlorothiophene-2-carboxylate (2.10 g, 60%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.49 (s, 9 H), 3.87 (s, 3 H), 4.83 (s, 2 H), 6.73 (dd, J = 8.08, 2.27 Hz, 1 H), 6.99 (bs, 1 H), 7.05 (d, J = 7.58 Hz, 1 H), 7.22 (t, J = 7.96 Hz, 1 H).

A solution of methyl 5-(3-aminophenyl)-3-(2-*tert*-butoxy-2-oxoethoxy)-4-chlorothiophene-2-carboxylate (212 mg, 0.53 mmol) and 1-(benzylsulfonyl)piperidin-4-one (190 mg, 0.74 mmol) in 1,2-dichloroethane (5 mL) was diluted with AcOH (50 mL). Sodium triacetoxyborohydride (340 mg, 1.59 mmol) was added to the solution, and the reaction was stirred at room temperature for 3 h. Solvents were evaporated, and the crude product was dissolved with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and filtered. The crude product was purified by flash column chromatography (30% EtOAc/hexane as eluent) to provide methyl 5-(3-{[1-(benzylsulfonyl)piperidin-4-yl]-amino}phenyl)-3-(2-*tert*-butoxy-2-oxoethoxy)-4-chlorothiophene-2-carboxylate (201 mg, 60%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.50 (s, 9 H), 1.95−2.09 (m, 2 H), 2.70−2.87 (m, 2 H), 3.35 (br. s., 1 H), 3.54−3.68 (m, J = 12.88 Hz, 3 H), 3.88 (bs, 3 H), 4.24 (s, 2 H), 4.83 (s, 2 H), 6.60 (dd, J = 7.83, 2.02 Hz, 1 H), 6.83−6.87 (m, J = 1.77 Hz, 1 H), 6.97 (d, J = 8.34 Hz, 1 H), 7.22 (t, J = 7.83 Hz, 1 H), 7.37−7.43 (m, 5 H).

A solution of methyl 5-(3-{[1-(benzylsulfonyl)piperidin-4-yl]-amino}phenyl)-3-(2-*tert*-butoxy-2-oxoethoxy)-4-chlorothiophene-2-carboxylate (201 mg, 0.31 mmol) and LiOH monohydrate (44 mg, 0.93 mmol) in THF (2 mL), MeOH (2 mL), and water (1 mL) was stirred at room temperature for 6 h. The volatiles were removed under vacuum, and the residue was diluted with water (10 mL) and acidified with 10% aqueous HCl. The resulting precipitate was collected by filtration and dried under vacuum to provide 5-(3-{-[1-(benzylsulfonyl)piperidin-4-yl]amino}phenyl)-3-(carboxymethoxy)-4-chlorothiophene-2-carboxylic acid (82 mg, 46%) as a tan solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.27−1.46 (m, 2 H), 1.94 (d, J = 9.85 Hz, 2 H), 2.92 (t, J = 10.99 Hz, 2 H), 3.53 (d, J = 12.88 Hz, 2 H), 4.40 (s, 2 H), 4.92 (s, 2 H), 6.71 (d, J = 8.84 Hz, 1 H), 6.82 (d, J = 7.83 Hz, 1 H), 6.87 (s, 1 H), 7.21 (t, J = 7.96 Hz, 1 H), 7.32−7.46 (m, 5 H). HRMS: calcd for $C_{25}H_{25}ClN_2O_7S_2 + H^+$, 565.086 45; found (ESI-FTMS, $[M + H]^+$), 565.087 23. RF-HPLC: H_2O/MeCN/0.1% formic acid, 94%; H_2O/MeCN/0.1% formic acid, 95%.

5-(3-{[1-(Benzylsulfonyl)piperidin-4-yl]oxy}phenyl)-4-bromo-3-(carboxymethoxy)thiophene-2-carboxylic Acid (36). To a solution of 3-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)phenol (441 mg, 2.0 mmol), 4-hydroxypiperidine-1-carboxylic acid *tert*-butyl ester (603.8 mg, 3.0 mmol), and triphenylphosphine (788 mg, 3.0 mmol) in THF (5 mL) was added DIAD (0.59 mL, 3.0 mmol) dropwise at 0 °C. The resulting solution was stirred at room temperature for 36 h. The solvent was removed and the crude product was purified by flash column chromatography to give 4-[3-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)phenoxy]piperidine-1-carboxylic acid *tert*-butyl ester (635 mg, 79%) as a colorless crystalline. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.33 (m, 12 H), 1.48 (m, 9 H), 1.75 (m, 2 H), 1.89 (m, 2 H), 3.37 (m, 2 H), 3.67 (m, 2 H), 4.53 (m, 1 H), 7.01 (m, 1 H), 7.29 (m, 1 H), 7.34 (d, J = 2.78 Hz, 1 H), 7.40 (m, 1 H).

4-[3-(3-Bromo-4-ethoxycarbonylmethoxy-5-methoxycarbonylthiophen-2-yl)phenoxy]piperidine-1-carboxylic acid *tert*-butyl ester (125 mg, 51%) was prepared according to the general procedure for Suzuki coupling, using 4,5-dibromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (212 mg, 0.53 mmol) and 4-[3-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)phenoxy]piperidine-1-carboxylic acid *tert*-butyl ester (165 mg, 0.41 mmol) as the starting materials. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.5 (s, 9 H), 1.8 (m, 2 H), 2.0 (m, 2 H), 3.4 (m, 2 H), 3.7 (m, 2 H), 4.5 (m, 1 H), 6.9 (dd, J = 7.7, 2.1 Hz, 1 H), 7.1 (m, 1 H), 7.2 (m, 1 H), 7.3 (t, J = 8.0 Hz, 1 H).

4-Bromo-3-ethoxycarbonylmethoxy-5-[3-(piperidin-4-yloxy)phenyl]thiophene-2-carboxylic acid methyl ester (TFA salt) was obtained by treating 4-[3-(3-bromo-4-ethoxycarbonylmethoxy-5-methoxycarbonylthiophen-2-yl)phenoxy]piperidine-1-carboxylic acid *tert*-butyl ester (210 mg, 0.35 mmol) with TFA/CH_2Cl_2 (v/v 10:1) at room temperature overnight.

4-Bromo-3-ethoxycarbonylmethoxy-5-[3-(1-phenylmethanesulfonylpiperidin-4-yloxy)phenyl]thiophene-2-carboxylic acid methyl ester (62 mg, 81%) was prepared as colorless oil according to the general procedure for sulfonamide formation at the piperidine nitrogen, using 4-bromo-3-ethoxycarbonylmethoxy-5-[3-(piperidin-4-yloxy)phenyl]thiophene-2-carboxylic acid methyl ester TFA salt (70 mg, 0.117 mmol) as the starting material.

Compound **36**, 5-(3-{[1-(benzylsulfonyl)piperidin-4-yl]oxy}-phenyl)-4-bromo-3-(carboxymethoxy)thiophene-2-carboxylic acid (43 mg, 57%), was prepared as a white solid according to the general procedure for hydrolysis, using 4-bromo-3-ethoxycarbonylmethoxy-5-[3-(1-phenylmethanesulfonylpiperidin-4-yloxy)phenyl]-thiophene-2-carboxylic acid methyl ester (60 mg, 0.092 mmol) as the starting material. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.7 (m, 2 H), 1.9 (m, 2 H), 3.1 (m, 2 H), 3.4 (m, 2 H), 4.4 (s, 2 H), 4.6 (m, 1 H), 4.8 (s, 2 H), 7.1 (m, 1 H), 7.2 (m, 2 H), 7.4 (m, 6 H).

5-[3-({[1-(Benzylsulfonyl)piperidin-4-yl]methyl}amino)phenyl]-4-bromo-3-(carboxymethoxy)thiophene-2-carboxylic Acid (37). *tert*-Butyl 4-((3-(3-bromo-4-(2-*tert*-butoxy-2-oxoethoxy)-5-(methoxycarbonyl)thiophen-2-yl)phenylamino)methyl)piperidine-1-carboxylate was prepared by following the general procedure of reductive amination, using 5-(3-aminophenyl)-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester (830 mg, 1.9 mmol), *tert*-butyl 4-formylpiperidine-1-carboxylate (400 mg, 1.9 mmol), acetic acid (0.16 mL, 2.9 mmol), and $NaBH(OAc)_3$ as starting materials. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.13−1.22 (m, 3 H), 1.46 (s, 9 H), 1.51 (s, 9 H), 1.74−1.83 (m, 4 H), 2.70 (t, J = 13.52 Hz, 2 H), 3.07 (t, J = 6.82 Hz, 2 H), 3.87 (s, 3 H), 4.82 (s, 2 H), 6.65 (ddd, J = 7.07, 1.52, 1.26 Hz, 1 H), 6.85−6.87 (m, 1 H), 6.93−6.97 (m, 1 H), 7.23 (t, J = 7.83 Hz, 1 H).

tert-Butyl 4-((3-(3-bromo-4-(2-*tert*-butoxy-2-oxoethoxy)-5-(methoxycarbonyl)thiophen-2-yl) phenylamino)methyl)piperidine-1-carboxylate (973 mg, 1.5 mmol) was dissolved in 1 N HCl/EtOAc (7.6 mmol) and MeOH (1.5 mL) and the reaction mixture was stirred at room temperature. A thick slurry resulted, which required the addition of 5 mL of EtOAc to aid in stirring. The mixture was allowed to stir overnight and then evaporated to give methyl 4-bromo-3-(2-*tert*-butoxy-2-oxoethoxy)-5-(3-(piperidin-4-ylmethylamino)phenyl)thiophene-2-carboxylate hydrochloride (837 mg, 97%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.49 (s, 9 H), 1.53−1.64 (m, 2 H), 2.06−2.20 (m, 3 H), 3.03 (td, J = 12.95, 2.40 Hz, 2 H), 3.37 (d, J = 6.57 Hz, 2 H), 3.41−3.48 (m, 2 H), 3.86 (s, 3 H), 4.86 (s, 2 H), 7.37−7.43 (m, 1 H), 7.50−7.61 (m, 3 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-{3-[(1-phenylmethanesulfonylpiperidin-4-ylmethyl)amino]phenyl}thiophene-2-carboxy-

lic acid methyl ester was prepared in 79% yield according to the general procedure for sulfonamide formation at the piperidine nitrogen, using 4-bromo-3-(2-*tert*-butoxy-2-oxoethoxy)-5-(3-(piperidin-4-ylmethylamino)phenyl)thiophene-2-carboxylate hydrochloride (57 mg, 0.1 mmol) and phenylmethanesulfonyl chloride (23 mg, 0.12 mmol) as starting materials. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.24 (m, 2 H), 1.51 (s, 9 H), 1.67 (m, 1 H), 1.76 (m, 2 H), 2.54 (td, J = 12.32, 2.40 Hz, 2 H), 3.03 (d, J = 6.82 Hz, 2 H), 3.69 (d, J = 12.38 Hz, 2 H), 3.87 (s, 3 H), 4.21 (s, 2 H), 4.82 (s, 2 H), 6.62 (dd, J = 7.83, 2.02 Hz, 1 H), 6.83 (m, 1 H), 6.94 (d, J = 8.08 Hz, 1 H), 7.22 (t, J = 7.83 Hz, 1 H), 7.38 (m, 5 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-{3-[(1-phenylmethanesulfonylpiperidin-4-ylmethyl)amino]phenyl}thiophene-2-carboxylic acid methyl ester was hydrolyzed according to the general procedure for hydrolysis to give compound **37** in 75% yield as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.14 (m, 2 H), 1.66 (m, 1 H), 1.79 (d, J = 12.38 Hz, 2 H), 2.67 (m, 2 H), 2.94 (d, J = 6.57 Hz, 2 H), 3.57 (d, J = 12.38 Hz, 2 H), 4.38 (s, 2 H), 4.88 (s, 2 H), 6.68 (dd, J = 8.97, 2.40 Hz, 1 H), 6.77 (d, J = 6.82 Hz, 1 H), 6.81 (s, 1 H), 7.18 (t, J = 7.96 Hz, 1 H), 7.39 (m, 5 H). HRMS: calcd for $C_{26}H_{27}BrN_2O_7S_2 + H^+$, 623.051 59; found (ESI-FTMS, $[M + H]^+$), 623.052 43. RF-HPLC: H_2O/MeCN/0.1% formic acid, 97%; H_2O/MeCN/0.1% formic acid, 98%.

5-{3-[(1-Benzenesulfonylpiperidin-4-ylmethyl)amino]phenyl}-4-bromo-3-carboxymethoxythiophene-2-carboxylic Acid (38). 5-{3-[(1-Benzenesulfonylpiperidin-4-ylmethyl)amino]phenyl}-4-bromo-3-*tert*-butoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was prepared in 60% yield according to the general procedure for sulfonamide formation at the piperidine nitrogen, using 4-bromo-3-(2-*tert*-butoxy-2-oxoethoxy)-5-(3-(piperidin-4-ylmethylamino)phenyl)thiophene-2-carboxylate hydrochloride (57 mg, 0.1 mmol) and benzenesulfonyl chloride (15 μL, 0.12 mmol) as starting materials. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.38 (m, 2 H), 1.50 (s, 9 H), 1.56 (m, 1 H), 1.85 (d, J = 10.86 Hz, 2 H), 2.26 (td, J = 11.94, 2.40 Hz, 2 H), 3.04 (d, J = 6.57 Hz, 2 H), 3.83 (m, 2 H), 3.87 (s, 3 H), 4.81 (s, 2 H), 6.60 (m, 1 H), 6.80 (m, 1 H), 6.93 (dd, J = 7.96, 1.39 Hz, 1 H), 7.20 (t, J = 7.83 Hz, 1 H), 7.52 (m, 2 H), 7.59 (m, 1 H), 7.76 (m, 2 H). 5-{3-[(1-Benzenesulfonylpiperidin-4-ylmethyl)amino]phenyl}-4-bromo-3-*tert*-butoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was hydrolyzed according to the general procedure for hydrolysis to give compound **38** in 82% yield as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.25 (m, 2 H), 1.52 (m, 1 H), 1.82 (m, 2 H), 2.21 (m, 2 H), 2.90 (d, J = 6.06 Hz, 2 H), 3.67 (d, J = 11.87 Hz, 2 H), 4.86 (s, 2 H), 6.64 (dd, J = 7.96, 1.89 Hz, 1 H), 6.77 (m, 2 H), 7.15 (t, J = 7.83 Hz, 1 H), 7.63 (m, 2 H), 7.71 (m, 3 H). HRMS: calcd for $C_{25}H_{25}BrN_2O_7S_2 + H^+$, 609.035 94; found (ESI-FTMS, $[M + H]^+$), 609.035 63. RF-HPLC: H_2O/MeCN/0.1% formic acid, 98%; H_2O/MeCN/0.1% formic acid, 99%.

5-[3-({[1-(Anilinocarbonyl)piperidin-4-yl]methyl}amino)-phenyl]-4-bromo-3-(carboxymethoxy)thiophene-2-carboxylic Acid (39). 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-{3-[(1-phenylcarbamoylpiperidin-4-ylmethyl)amino]phenyl}thiophene-2-carboxylic acid methyl ester was obtained in 62% yield by following the general procedure for urea formation at the piperidine nitrogen, using methyl 4-bromo-3-(2-*tert*-butoxy-2-oxoethoxy)-5-(3-(piperidin-4-ylmethylamino)phenyl)thiophene-2-carboxylate hydrochloride (57 mg, 0.1 mmol), DIPEA (27 μL, 0.15 mmol), and phenyl isocyanate (12 μL, 0.11 mmol) as starting materials. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.31 (m, 2 H), 1.51 (s, 9 H), 1.88 (m, 3 H), 2.89 (m, 2 H), 3.10 (d, J = 6.06 Hz, 2 H), 3.87 (s, 3 H), 4.12 (m, 2 H), 4.82 (s, 2 H), 6.38 (s, 1 H), 6.66 (m, 1 H), 6.87 (m, 1 H), 6.96 (dd, J = 6.69, 1.64 Hz, 1 H), 7.03 (m, 1 H), 7.24 (m, 1 H), 7.29 (m, 2 H), 7.35 (m, 2 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-{3-[(1-phenylcarbamoylpiperidin-4-ylmethyl)amino]phenyl}thiophene-2-carboxylic acid methyl ester (41 mg, 0.062 mmol) was hydrolyzed according to the general procedure for hydrolysis to give compound **39** as a pale yellow solid in 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.16 (m, 2 H), 1.79 (m, 3 H), 2.77 (m, 2 H), 2.97 (d, J = 5.81 Hz, 2 H), 4.15 (d, J = 12.63 Hz, 2 H), 4.87 (s, 2 H), 6.70 (dd, J = 8.72, 1.89 Hz, 1 H), 6.78 (d, J = 7.58 Hz, 1 H), 6.84 (t, J = 1.77 Hz, 1 H), 6.91 (t, J = 7.33 Hz, 1 H), 7.20 (m, 3 H), 7.45 (dd, J = 8.72, 1.14 Hz, 2 H), 8.44 (s, 1 H). HRMS: calcd for $C_{26}H_{26}BrN_3O_6S + H^+$, 588.079 84; found (ESI-FTMS, $[M + H]^+$), 588.079 91.

5-(3-{[1-(Anilinocarbonyl)piperidin-4-yl]methoxy}phenyl)-4-bromo-3-(carboxymethoxy)thiophene-2-carboxylic Acid (40). To a solution of 4-bromo-3-ethoxycarbonylmethoxy-5-(3-hydroxyphenyl)thiophene-2-carboxylic acid methyl ester in DMF (4 mL) was added 4-methanesulfonyloxypiperidine-1-carboxylic acid *tert*-butyl ester (2 equiv) and K_2CO_3 (excess). The mixture was stirred at 80 °C overnight. The solution was then washed with saturated $NaHCO_3$ and dried over Na_2SO_4. The crude product was purified by flash column chromatography to give 4-[3-(3-bromo-4-*tert*-butoxycarbonylmethoxy-5-methoxycarbonylthiophen-2-yl)phenoxymethyl]piperidine-1-carboxylic acid *tert*-butyl ester (84 mg, 39%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.3 (m, 2 H), 1.5 (s, 9 H), 1.5 (s, 9 H), 1.8 (m, 2 H), 2.0 (m, 1 H), 2.8 (t, J = 12.3 Hz, 2 H), 3.8 (d, J = 6.6 Hz, 2 H), 3.9 (s, 3 H), 4.8 (s, 2 H), 7.0 (dd, 1 H), 7.2 (t, 1 H), 7.2 (dt, J = 8.3 Hz, 1 H), 7.3 (t, J = 8.0 Hz, 1 H).

4-Bromo-3-ethoxycarbonylmethoxy-5-[3-(piperidin-4-ylmethoxy)phenyl]thiophene-2-carboxylic acid methyl ester TFA salt was obtained by treating 4-[3-(3-bromo-4-*tert*-butoxycarbonylmethoxy-5-methoxycarbonylthiophen-2-yl)phenoxymethyl]piperidine-1-carboxylic acid *tert*-butyl ester with 30% TFA in DCM at room temperature.

4-Bromo-3-ethoxycarbonylmethoxy-5-[3-(1-phenylcarbamoylpiperidin-4-ylmethoxy)phenyl]thiophene-2-carboxylic acid methyl ester (90% yield) was prepared according to the general procedure for urea formation at the piperidine nitrogen. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.2 (t, J = 7.2 Hz, 3 H), 1.4 (m, 2 H), 1.7 (m, 1 H), 1.8 (m, 2 H), 2.3 (td, J = 12.0, 2.5 Hz, 2 H), 3.7 (d, J = 6.3 Hz, 2 H), 3.8 (s, 3 H), 4.2 (q, J = 7.3 Hz, 2 H), 4.8 (s, 2 H), 6.8 (dd, 1 H), 7.1 (t, 1 H), 7.1 (m, 1 H), 7.3 (t, J = 8.0 Hz, 1 H), 7.5 (m, 3 H), 7.7 (m, 2 H).

4-Bromo-3-ethoxycarbonylmethoxy-5-[3-(1-phenylcarbamoylpiperidin-4-ylmethoxy)phenyl]thiophene-2-carboxylic acid methyl ester was hydrolyzed according to the general procedure for hydrolysis to give compound **40** in 75% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.0 (d, 2 H), 2.0 (td, 2 H), 2.8 (m, 2 H), 3.0 (d, 2 H), 3.3 (d, 2 H), 4.5 (m, 2 H), 6.1 (m, 2 H), 6.3 (m, 4 H), 6.4 (m, 3 H). HRMS: calcd for $C_{26}H_{25}BrN_2O_7S + H^+$, 589.063 86; found (ESI-FTMS, $[M + H]^+$), 589.064 26.

4-Bromo-3-(carboxymethoxy)-5-[3-({1-[(4-chlorobenzyl)sulfonyl]piperidin-4-yl}amino)phenyl]thiophene-2-carboxylic Acid (41). 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-{3-[1-(4-chlorophenylmethanesulfonyl)piperidin-4-ylamino]phenyl}thiophene-2-carboxylic acid methyl ester (108 mg, 77%) was prepared according to the general procedure for sulfonamide formation at the piperidine nitrogen, using 4-bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(piperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester HCl salt (110 mg, 0.20 mmol) as the starting material. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.43 (m, 2 H), 1.51 (s, 9 H), 2.08 (dd, J = 13.52, 2.91 Hz, 2 H), 2.82 (m, 2 H), 3.39 (m, 1 H), 3.65 (m, 3 H), 3.88 (s, 3 H), 4.18 (s, 2 H), 4.82 (s, 2 H), 6.63 (m, 1 H), 6.84 (m, 1 H), 6.95 (d, J = 7.58 Hz, 1 H), 7.22 (t, J = 7.83 Hz, 1 H), 7.37 (m, 4 H).

Compound **41**, 4-bromo-3-carbonylmethoxy-5-{3-[1-(4-chlorophenylmethanesulfonyl)piperidin-4-ylamino]phenyl}thiophene-2-carboxylic acid (55 mg, 98%), was prepare as a yellow solid according to the general procedure for hydrolysis, using 4-bromo-3-*tert*-butoxycarbonylmethoxy-5-{3-[1-(4-chlorophenylmethanesulfonyl)piperidin-4-ylamino]phenyl}thiophene-2-carboxylic acid methyl ester (62 mg, 0.087 mmol) as the starting material. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.36 (m, 2 H), 1.96 (d, J = 13.89 Hz, 2 H), 2.93 (t, J = 9.85 Hz, 2 H), 3.33 (s, 1 H), 3.53 (m, 2 H), 4.43 (s, 2 H), 4.87 (s, 2 H), 6.71 (dd, J = 8.59, 2.02 Hz, 1 H), 6.79 (m, 1 H), 6.85 (m, 1 H), 7.20 (t, J = 7.96 Hz, 1 H), 7.45 (d, J = 2.53 Hz, 4 H). HRMS: calcd for $C_{25}H_{24}BrClN_2O_7S_2 + H^+$, 642.996 96; found (ESI-FTMS, $[M + H]^+$), 642.997 07.

4-Bromo-3-(carboxymethoxy)-5-(3-(1-(3-chlorobenzylsulfonyl)-piperidin-4-ylamino)phenyl)thiophene-2-carboxylic Acid (42).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-{3-[1-(3-chlorophenylmethanesulfonyl)piperidin-4-ylamino]phenyl}thiophene-2-carboxylic acid methyl ester was prepared by following the general procedure for sulfonamide formation at the piperidine nitrogen to give a light yellow solid (110 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.45 (m, 2 H), 1.50 (d, J = 10.11 Hz, 9 H), 1.55 (m, 4 H), 2.09 (m, 2 H), 2.85 (m, 2 H), 3.39 (s, 1 H), 3.64 (m, 2 H), 3.88 (s, 3 H), 4.82 (s, 2 H), 6.64 (d, J = 2.02 Hz, 1 H), 6.85 (s, 1 H), 6.96 (d, J = 7.83 Hz, 1 H), 7.34 (m, 6 H).

4-Bromo-3-carboxymethoxy-5-{3-[1-(3-chlorophenylmethanesulfonyl)piperidin-4-ylamino]phenyl}thiophene-2-carboxylic acid was prepared as a yellow solid (81 mg, 90%) by following the general procedure for hydrolysis. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.39 (s, 2 H), 1.95 (d, J = 31.83 Hz, 2 H), 2.96 (s, 1 H), 3.56 (s, 1 H), 4.45 (s, 2 H), 4.87 (s, 2 H), 6.71 (s, 1 H), 6.79 (m, 1 H), 6.85 (t, J = 1.89 Hz, 1 H), 7.20 (t, J = 7.83 Hz, 1 H), 7.40 (d, J = 2.02 Hz, 1 H), 7.43 (m, 2 H), 7.50 (s, 1 H). HRMS: calcd for $C_{25}H_{24}BrClN_2O_7S_2 + H^+$, 641.989 68; found (ESI-FTMS, [M + H]$^+$), 641.989 24.

3-Carboxymethoxy-5-{3-[1-(2-chlorobenzenesulfonyl)piperidin-4-ylamino]phenyl}-4-methylthiophene-2-carboxylic Acid (43). Methyl 4-bromo-3-(2-*tert*-butoxy-2-oxoethoxy)-5-(3-(1-(2-chlorobenzylsulfonyl)piperidin-4-ylamino)phenyl)thiophene-2-carboxylate was prepared by following the general procedure for sulfonamide formation at the piperidine nitrogen to give a tan solid (112 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.49 (m, 9 H), 1.56 (m, 2 H), 2.13 (m, 2 H), 2.24 (s, 3 H), 2.98 (m, 2 H), 3.43 (m, 1 H), 3.62 (m, 1 H), 3.85 (m, 2 H), 3.85 (m, 3 H), 4.79 (d, 2 H), 6.58 (m, 1 H), 6.62 (m, 1 H), 6.79 (m, J = 7.58 Hz, 1 H), 7.20 (t, J = 7.83 Hz, 1 H), 7.41 (ddd, J = 8.21, 6.69, 1.52 Hz, 1 H), 7.50 (m, J = 7.58, 7.58, 1.52 Hz, 1 H), 7.53 (d, J = 1.52 Hz, 1 H), 8.08 (dd, J = 7.71, 1.89 Hz, 1 H).

Methyl 4-bromo-3-(2-*tert*-butoxy-2-oxoethoxy)-5-(3-(1-(2-chlorobenzylsulfonyl)piperidin-4-ylamino)phenyl)thiophene-2-carboxylate was hydrolyzed by following the general procedure for hydrolysis to give compound **43** (53 mg, 43%) as a beige solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.41 (d, J = 5.31 Hz, 2 H), 1.94 (s, 2 H), 2.17 (m, 3 H), 2.96 (s, 2 H), 3.65 (s, 2 H), 4.85 (s, 2 H), 5.87 (t, 1 H), 6.39 (m, 1 H), 6.63 (m, J = 6.95, 6.95 Hz, 2 H), 6.68 (s, 1 H), 7.15 (t, J = 7.83 Hz, 1 H), 7.58 (m, 1 H), 7.70 (m, 2 H), 7.99 (dd, J = 7.83, 1.52 Hz, 1 H). HRMS: calcd for $C_{25}H_{25}ClN_2O_7S_2 + H^+$, 565.086 50; found (ESI-FTMS, [M + H]$^+$), 565.086 53. RF-HPLC: H_2O/MeCN/0.1% formic acid, 98%; H_2O/MeCN/0.1% formic acid, 98%.

4-Bromo-3-(carboxymethoxy)-5-{3-[(1-{[2-(trifluoromethyl)-benzyl]sulfonyl}piperidin-4-yl)amino]phenyl}thiophene-2-carboxylic Acid (44). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.32−1.51 (m, 2 H), 1.93−2.09 (m, 2 H), 2.99−3.17 (m, 2 H), 3.42−3.54 (m, 1 H), 3.55−3.68 (m, 2 H), 4.53 (s, 3 H), 4.88 (s, 2 H), 6.74 (dd, J = 8.59, 1.77 Hz, 1 H), 6.81 (d, J = 7.33 Hz, 1 H), 6.87 (s, 1 H), 7.21 (t, J = 7.83 Hz, 1 H), 7.57−7.65 (m, 1 H), 7.67−7.76 (m, 2 H), 7.80 (d, J = 7.83 Hz, 1 H). HRMS: calcd for $C_{26}H_{24}BrF_3N_2O_7S_2 + H^+$, 677.023 31; found (ESI-FTMS, [M + H]$^+$), 677.0242.

4-Bromo-3-(carboxymethoxy)-5-[3-({1-[(2-methylbenzyl)sulfonyl]piperidin-4-yl}amino)phenyl]thiophene-2-carboxylic Acid (45). 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(1-*o*-tolylmethanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester was prepared by following the procedure in the synthesis of **32**, using 4-bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(piperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester (0.2 g, 0.38 mmol) and *o*-tolyl-methanesulfonyl chloride (0.24 g, 1.14 mmol) as starting materials. 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(1-*o*-tolylmethanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester (0.096 g, 40%) was obtained as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.51 (s, 9 H), 2.09 (dd, J = 12.88, 4.04 Hz, 2 H), 2.47 (s, 3 H), 2.89 (m, 2 H), 3.41 (m, 1 H), 3.67 (m, 4 H), 3.88 (s, 3 H), 4.28 (s, 2 H), 4.82 (s, 2 H), 6.63 (m, 1 H), 6.85 (m, 1 H), 6.95 (m, 1 H), 7.26 (m, 5 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(1-*o*-tolylmethanesulfonylpiperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester (0.096 g, 0.15 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-(carboxymethoxy)-5-[3-({1-[(2-methylbenzyl)sulfonyl]piperidin-4-yl}amino)-phenyl]thiophene-2-carboxylic acid (0.046 g, 49%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ ppm 1.41 (m, 2 H), 2.00 (m, 2 H), 2.35 (s, 3 H), 2.93 (t, J = 11.12 Hz, 2 H), 3.38 (m, 1 H), 3.60 (d, J = 12.88 Hz, 2 H), 4.27 (s, 2 H), 4.82 (s, 2H), 6.65 (dd, J = 8.84, 2.78 Hz, 1 H), 6.77 (dd, J = 6.82, 1.52 Hz, 1 H), 6.83 (t, J = 1.89 Hz, 1 H), 7.12 (m, 4 H), 7.25 (d, J = 7.07 Hz, 1 H). HRMS: calcd for $C_{26}H_{27}BrN_2O_7S_2 + H^+$, 623.051 58; found (ESI (+), [M + H]$^+$), 623.0529. RF-HPLC: H_2O/MeCN/0.1% formic acid, 95%; H_2O/MeCN/0.1% formic acid, 96%.

4-Bromo-3-(carboxymethoxy)-5-[3-({1-[(2,6-dimethylbenzyl)-sulfonyl]piperidin-4-yl}amino)phenyl]thiophene-2-carboxylic Acid (46). 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-{3-[1-(2,6-dimethylphenylmethanesulfonyl)piperidin-4-ylamino]phenyl}thiophene-2-carboxylic acid methyl ester was prepared by following the procedure in the synthesis of **32**, using 4-bromo-3-*tert*-butoxycarbonylmethoxy-5-[3-(piperidin-4-ylamino)phenyl]thiophene-2-carboxylic acid methyl ester (0.194 g, 0.35 mmol) and 2,6-dimethylbenzylsulfonyl chloride (0.24 g, 1.14 mmol) as starting materials. 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-{3-[1-(2,6-dimethylphenylmethanesulfonyl)piperidin-4-ylamino]phenyl}-thiophene-2-carboxylic acid methyl ester (0.091 g, 34%) was obtained as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.52 (m, 9 H), 1.59 (m, 2 H), 2.47 (s, 6 H), 2.96 (m, 2 H), 3.45 (m, 1 H), 3.77 (m, 2 H), 3.88 (s, 3 H), 4.35 (s, 2 H), 4.82 (s, 2 H), 6.74 (d, J = 8.08 Hz, 1 H), 6.97 (s, 1 H), 7.06 (m, J = 12.88, 7.58 Hz, 3 H), 7.15 (m, 1 H), 7.26 (m, 1 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-{3-[1-(2,6-dimethylphenylmethanesulfonyl)piperidin-4-ylamino]phenyl}thiophene-2-carboxylic acid methyl ester (0.091 g, 0.15 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-(carboxymethoxy)-5-[3-({1-[(2,6-dimethylbenzyl)sulfonyl]piperidin-4-yl}amino)phenyl]thiophene-2-carboxylic acid (0.043 g, 45%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ ppm 1.48 (m, 2 H), 2.06 (d, J = 10.36 Hz, 2 H), 2.36 (s, 6 H), 3.04 (t, J = 10.61 Hz, 2 H), 3.43 (m, J = 10.23, 10.23 Hz, 1 H), 3.69 (m, J = 12.63 Hz, 2 H), 4.34 (s, 2 H), 4.78 (s, 2 H), 6.68 (dd, J = 8.21, 1.64 Hz, 1 H), 6.79 (d, J = 8.08 Hz, 1 H), 6.85 (m, 1 H), 6.96 (m, J = 7.33 Hz, 2 H), 7.02 (m, 1 H), 7.12 (t, J = 7.83 Hz, 1 H). HRMS: calcd for $C_{27}H_{29}BrN_2O_7S_2 + H^+$, 637.067 23; found (ESI/FTMS, [M + H]$^+$), 637.0677. RF-HPLC: H_2O/MeCN/0.1% formic acid, 96%; H_2O/MeCN/0.1% formic acid, 95%.

5-[3-({1-[(2-Aminobenzyl)sulfonyl]piperidin-4-yl}amino)-phenyl]-4-bromo-3-(carboxymethoxy)thiophene-2-carboxylic Acid (47). 5-{3-[1-(2-Amino-phenylmethanesulfonyl)piperidin-4-ylamino]phenyl}-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was obtained in 19% yield as a pale yellow foam, according to the general procedure for sulfonamide formation at the piperidine nitrogen followed by $SnCl_2$ reduction. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.3 (t, J = 7.2 Hz, 3 H), 1.5 (m, 2 H), 2.1 (m, 2 H), 2.9 (m, 2 H), 3.4 (m, 1 H), 3.7 (m, 2 H), 3.9 (s, 3 H), 4.3 (m, 4 H), 4.9 (s, 2 H), 6.6 (m, 1 H), 6.8 (m, 2 H), 6.8 (m, 1 H), 6.9 (m, 1 H), 7.1 (m, 2 H), 7.2 (t, J = 7.8 Hz, 1 H), 7.3 (s, 1 H).

5-{3-[1-(2-Amino-phenylmethanesulfonyl)piperidin-4-ylamino]-phenyl}-4-bromo-3-ethoxycarbonylmethoxythiophene-2-carboxylic acid methyl ester was hydrolyzed according to the general procedure for hydrolysis to give compound **47** in 36% yield as a pale brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.4 (m, 2 H), 1.8 (m, J = 6.7, 6.7 Hz, 1 H), 2.0 (m, 2 H), 3.0 (m, 2 H), 3.6 (m, 2 H), 4.3 (s, 2 H), 4.9 (s, 2 H), 6.6 (m, 1 H), 6.8 (m, 3 H), 6.9 (m, 1 H), 7.1 (m, 1 H), 7.1 (dd, J = 7.7, 1.6 Hz, 1 H), 7.2 (t, J = 8.0 Hz, 1 H). HRMS: calcd for $C_{25}H_{26}BrN_3O_7S_2 + H^+$, 624.046 83; found (ESI-FTMS, [M + H]$^+$), 624.047 53.

5-{3-[(1-{[2-(Acetylamino)benzyl]sulfonyl}piperidin-4-yl)amino]phenyl}-4-bromo-3-(carboxymethoxy)thiophene-2-carboxylic Acid (48). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.27−1.49 (m, 2 H), 1.86−2.01 (m, 2 H), 2.08 (s, 3 H), 2.80−2.96 (m, 2 H), 3.32−3.44 (m, 1 H), 3.46−3.55 (m, 2 H), 4.50 (s, 2 H), 6.74 (d, J = 6.82 Hz, 1 H), 6.83 (d, J = 8.34 Hz, 1 H), 6.88 (s, 1 H), 7.14−

7.28 (m, 2 H), 7.29−7.38 (m, 1 H), 7.43 (dd, J = 7.58, 1.52 Hz, 1 H), 7.50 (d, J = 8.34 Hz, 1 H), 9.44 (s, 1 H). HRMS: calcd for $C_{27}H_{28}BrN_3O_8S_2 - H^+$, 664.042 84; found (ESI-FTMS, [M − H]−), 664.0423. RF-HPLC: H_2O/MeCN/0.1% formic acid, 95%; H_2O/MeCN/0.1% formic acid, 96%.

4-Bromo-3-(carboxymethoxy)-5-[3-({1-[(2-{[(ethylamino)carbonyl]amino}benzyl)sulfonyl]piperidin-4-yl}amino)phenyl]thiophene-2-carboxylic Acid (49). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.05 (t, J = 7.20 Hz, 3 H), 1.26−1.46 (m, 2 H), 1.93 (d, J = 9.85 Hz, 2 H), 2.91 (t, J = 10.61 Hz, 2 H), 3.06−3.18 (m, J = 6.95, 4.42 Hz, 2 H), 3.29−3.44 (m, 1 H), 3.44−3.58 (m, 2 H), 4.42 (s, 2 H), 6.63 (t, J = 4.42 Hz, 1 H), 6.71 (dd, J = 8.34, 1.77 Hz, 1 H), 6.79 (d, J = 7.58 Hz, 1 H), 6.84 (d, J = 1.77 Hz, 1 H), 6.99−7.09 (m, 1 H), 7.20 (t, J = 7.83 Hz, 1 H), 7.24−7.30 (m, 1 H), 7.35 (dd, J = 7.83, 1.52 Hz, 1 H), 7.70 (dd, J = 8.21, 1.14 Hz, 1 H), 7.77 (s, 1 H). HRMS: calcd for $C_{28}H_{31}BrN_4O_8S_2 - H^+$, 693.069 39; found (ESI-FTMS, [M − H]−), 693.071. RF-HPLC: H_2O/MeCN/0.1% formic acid, 99%; H_2O/MeCN/0.1% formic acid, 99%.

4-Bromo-3-(carboxymethoxy)-5-(3-{[1-({2-[(methylsulfonyl)amino]benzyl}sulfonyl)piperidin-4-yl]amino}phenyl)thiophene-2-carboxylic Acid (50). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.27−1.48 (m, 2 H), 1.88−2.05 (m, 2 H), 2.86−3.01 (m, 2 H), 3.02 (s, 3 H), 3.35−3.48 (m, 1 H), 3.48−3.58 (m, 2 H), 4.60 (s, 2 H), 6.72 (d, J = 7.58 Hz, 1 H), 6.80 (d, J = 7.58 Hz, 1 H), 6.86 (s, 1 H), 7.21 (t, J = 7.96 Hz, 1 H), 7.27−7.35 (m, 1 H), 7.36−7.47 (m, 3 H), 7.51 (dd, J = 7.83, 1.52 Hz, 1 H), 9.14 (s, 1 H). HRMS: calcd for $C_{26}H_{28}BrN_3O_9S_3 - H^+$, 700.009 83; found (ESI-FTMS, [M − H]−), 700.0084. RF-HPLC: H_2O/MeCN/0.1% formic acid, 98%; H_2O/MeCN/0.1% formic acid, 99%.

4-Bromo-3-carboxymethoxy-5-(3-{[1-(2-chlorophenylcarbamoyl)-piperidin-4-ylmethyl]amino}phenyl)thiophene-2-carboxylic Acid (51). 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-(3-{[1-(2-chlorophenylcarbamoyl)piperidin-4-ylmethyl]amino}phenyl)thiophene-2-carboxylic acid methyl ester was obtained in 56% yield by following the general procedure for urea formation at the piperidine nitrogen, using 4-bromo-3-(2-*tert*-butoxy-2-oxoethoxy)-5-(3-(piperidin-4-ylmethylamino)phenyl)thiophene-2-carboxylate hydrochloride (86 mg, 0.15 mmol), 2-chlorophenyl isocyanate (20 μL, 0.17 mmol), and DIPEA (67 μL, 0.38 mmol) as starting materials. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.33 (dd, J = 12.25, 2.91 Hz, 2 H), 1.51 (s, 9 H), 1.91 (m, 3 H), 2.94 (m, 2 H), 3.10 (d, J = 6.06 Hz, 2 H), 3.87 (s, 3 H), 4.15 (m, 2 H), 4.82 (s, 2 H), 6.66 (m, 1 H), 6.87 (m, 1 H), 6.94 (m, 2 H), 7.04 (s, 1 H), 7.23 (m, 2 H), 7.33 (dd, J = 8.08, 1.52 Hz, 1 H), 8.18 (dd, J = 8.34, 1.52 Hz, 1 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-(3-{[1-(2-chlorophenylcarbamoyl)piperidin-4-ylmethyl]amino}phenyl)thiophene-2-carboxylic acid methyl ester (58 mg, 0.084 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-(3-{[1-(2-chlorophenylcarbamoyl)piperidin-4-ylmethyl]amino}phenyl)thiophene-2-carboxylic acid (46 mg, 88%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.16 (m, 2 H), 1.79 (m, 3 H), 2.82 (m, 2 H), 2.97 (d, J = 6.82 Hz, 2 H), 4.11 (d, J = 13.39 Hz, 2 H), 4.87 (s, 2 H), 6.70 (dd, J = 8.21, 2.15 Hz, 1 H), 6.78 (d, J = 7.58 Hz, 1 H), 6.84 (t, J = 1.77 Hz, 1 H), 7.12 (td, J = 7.64, 1.64 Hz, 1 H), 7.19 (t, J = 7.83 Hz, 1 H), 7.27 (td, J = 7.71, 1.52 Hz, 1 H), 7.43 (dd, J = 7.96, 1.39 Hz, 1 H), 7.49 (dd, J = 7.96, 1.39 Hz, 1 H), 8.10 (s, 1 H). HRMS: calcd for $C_{26}H_{25}BrClN_3O_6S - H^+$, 622.040 88; found (ESI-FTMS, [M − H]−), 622.042 17. RF-HPLC: H_2O/MeCN/0.1% formic acid, 96%; H_2O/MeCN/0.1% formic acid, 96%.

4-Bromo-3-carboxymethoxy-5-(3-{[1-(2-methoxyphenylcarbamoyl)piperidin-4-ylmethyl]amino}phenyl)thiophene-2-carboxylic Acid (52). 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-(3-{[1-(2-methoxyphenylcarbamoyl)piperidin-4-ylmethyl]amino}phenyl)thiophene-2-carboxylic acid methyl ester was obtained in 56% yield by following the general procedure for urea formation at the piperidine nitrogen, using 4-bromo-3-(2-*tert*-butoxy-2-oxoethoxy)-5-(3-(piperidin-4-ylmethylamino)phenyl)thiophene-2-carboxylate hydrochloride (86 mg, 0.15 mmol), 2-methoxyphenyl isocyanate (22 μL, 0.17 mmol), and DIPEA (67 μL, 0.38 mmol) as starting materials. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.33 (m, 2 H), 1.51 (s, 9 H), 1.87 (dd, J = 11.37, 8.59 Hz, 3 H), 2.90 (m, 2 H), 3.10 (d, J = 6.32 Hz, 2 H), 3.87 (s, 3 H), 3.88 (s, 3 H), 4.14 (d, J = 13.14 Hz, 2 H), 4.82 (s, 2 H), 6.66 (m, 1 H), 6.86 (m, 2 H), 6.94 (m, 3 H), 7.13 (s, 1 H), 7.24 (t, J = 7.96 Hz, 1 H), 8.14 (m, 1 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-(3-{[1-(2-methoxyphenylcarbamoyl)piperidin-4-ylmethyl]amino}phenyl)thiophene-2-carboxylic acid methyl ester (58 mg, 0.084 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-(3-{[1-(2-methoxyphenylcarbamoyl)piperidin-4-ylmethyl]amino}phenyl)thiophene-2-carboxylic acid (48 mg, 92%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.16 (m, 2 H), 1.80 (m, 3 H), 2.81 (m, 2 H), 2.97 (d, J = 5.81 Hz, 2 H), 3.80 (s, 3 H), 4.07 (d, J = 13.39 Hz, 2 H), 4.87 (s, 2 H), 6.70 (m, 1 H), 6.77 (d, J = 7.83 Hz, 1 H), 6.85 (m, 2 H), 6.98 (m, 2 H), 7.19 (t, J = 7.83 Hz, 1 H), 7.57 (s, 1 H), 7.66 (d, J = 7.58 Hz, 1 H). HRMS: calcd for $C_{27}H_{28}BrN_3O_7S - H^+$, 618.090 41; found (ESI-FTMS, [M − H]−), 618.091 51. RF-HPLC: H_2O/MeCN/0.1% formic acid, 96%; H_2O/MeCN/0.1% formic acid, 95%.

4-Bromo-3-carboxymethoxy-5-{3-[(1-*o*-tolylcarbamoylpiperidin-4-ylmethyl)amino]phenyl}thiophene-2-carboxylic Acid (53). 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-{3-[(1-*o*-tolylcarbamoylpiperidin-4-ylmethyl)amino]phenyl}thiophene-2-carboxylic acid methyl ester was obtained in 41% yield by following the general procedure for urea formation at the piperidine nitrogen, using 4-bromo-3-(2-*tert*-butoxy-2-oxoethoxy)-5-(3-(piperidin-4-ylmethylamino)phenyl)thiophene-2-carboxylate hydrochloride (86 mg, 0.15 mmol), *o*-tolyl isocyanate (20 μL, 0.17 mmol), and DIPEA (67 μL, 0.38 mmol) as starting materials. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.33 (m, 2 H), 1.51 (s, 9 H), 1.88 (m, 3 H), 2.25 (s, 3 H), 2.91 (td, J = 12.88, 2.02 Hz, 2 H), 3.10 (d, J = 5.81 Hz, 2 H), 3.87 (s, 3 H), 3.95 (s, 1 H), 4.11 (d, J = 13.14 Hz, 2 H), 4.82 (s, 2 H), 6.15 (s, 1 H), 6.66 (m, 1 H), 6.87 (m, 1 H), 6.96 (m, 1 H), 7.01 (td, J = 7.45, 1.26 Hz, 1 H), 7.18 (m, 2 H), 7.24 (t, J = 7.96 Hz, 1 H), 7.62 (d, J = 8.08 Hz, 1 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-{3-[(1-*o*-tolylcarbamoylpiperidin-4-ylmethyl)amino]phenyl}thiophene-2-carboxylic acid methyl ester (43 mg, 0.064 mmol) was hydrolyzed according to the general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-{3-[(1-*o*-tolylcarbamoylpiperidin-4-ylmethyl)amino]phenyl}thiophene-2-carboxylic acid (34 mg, 88%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.17 (m, 2 H), 1.78 (m, 3 H), 2.15 (s, 3 H), 2.78 (m, 2 H), 2.97 (d, J = 6.06 Hz, 2 H), 4.11 (d, J = 13.39 Hz, 2 H), 4.88 (s, 2 H), 6.70 (dd, J = 8.21, 1.64 Hz, 1 H), 6.78 (d, J = 8.34 Hz, 1 H), 6.84 (t, J = 1.89 Hz, 1 H), 7.02 (td, J = 7.26, 1.64 Hz, 1 H), 7.10 (m, 1 H), 7.18 (m, 3 H), 7.97 (s, 1 H). HRMS: calcd for $C_{27}H_{28}BrN_3O_6S - H^+$, 602.0955; found (ESI-FTMS, [M − H]−), 602.097 88. RF-HPLC: H_2O/MeCN/0.1% formic acid, 93%; H_2O/MeCN/0.1% formic acid, 94%.

4-Bromo-3-carboxymethoxy-5-(3-{[1-(2,6-dimethylphenylcarbamoyl)piperidin-4-ylmethyl]amino}phenyl)thiophene-2-carboxylic Acid (54). 4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-(3-{[1-(2,6-dimethylphenylcarbamoyl)piperidin-4-ylmethyl]amino}phenyl)thiophene-2-carboxylic acid methyl ester was obtained in 69% yield by following the general procedure for urea formation at the piperidine nitrogen, using 4-bromo-3-(2-*tert*-butoxy-2-oxoethoxy)-5-(3-(piperidin-4-ylmethylamino)phenyl)thiophene-2-carboxylate hydrochloride (57 mg, 0.1 mmol), 2,6-dimethylphenyl isocyanate (17 μL, 0.12 mmol), and DIPEA (45 μL, 0.25 mmol) as starting materials. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.30 (m, 3 H), 1.51 (s, 9 H), 1.86 (d, J = 9.60 Hz, 2 H), 2.24 (s, 6 H), 2.90 (m, 2 H), 3.10 (d, J = 6.32 Hz, 2 H), 3.87 (s, 3 H), 4.10 (m, 2 H), 4.82 (s, 2 H), 5.82 (s, 1 H), 6.66 (m, 1 H), 6.87 (m, 1 H), 6.96 (d, J = 8.34 Hz, 1 H), 7.24 (m, 1 H).

4-Bromo-3-*tert*-butoxycarbonylmethoxy-5-(3-{[1-(2,6-dimethylphenylcarbamoyl)piperidin-4-ylmethyl]amino}phenyl)thiophene-2-carboxylic acid methyl ester was hydrolyzed according to the

general procedure for hydrolysis to give 4-bromo-3-carboxymethoxy-5-(3-{[1-(2,6-dimethylphenylcarbamoyl)piperidin-4-ylmethyl]amino}-phenyl)thiophene-2-carboxylic acid (35 mg, 82%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.14 (m, 2 H), 1.80 (m, 3 H), 2.13 (s, 6 H), 2.79 (m, 2 H), 2.97 (d, J = 6.06 Hz, 2 H), 4.12 (d, J = 12.63 Hz, 2 H), 4.88 (s, 2 H), 6.70 (dd, J = 8.34, 1.52 Hz, 1 H), 6.78 (d, J = 7.58 Hz, 1 H), 6.84 (t, J = 1.89 Hz, 1 H), 7.03 (m, 3 H), 7.19 (t, J = 7.96 Hz, 1 H). HRMS: calcd for $C_{28}H_{30}BrN_3O_6S - H^+$, 616.111 15; found (ESI-FTMS, [M − H]−), 616.112 83. RF-HPLC: H_2O/MeCN/0.1% formic acid, 92%; H_2O/MeCN/0.1% formic acid, 93%.

5.2. Enzymatic Assays. The enzymatic assays were carried out at room temperature in 96-well plates. The assay buffer contained 50 mM 3,3-dimethyl glutarate, 1 mM EDTA, 1 mM TCEP, and 0.01% Triton (pH 7.0 with ionic strength of 0.15 M adjusted by sodium chloride). The reaction was initiated by addition of the enzyme at a final concentration of 10 or 100 nM for PTP1B, 20 nM for TCPTP, 20 nM for CD45, and 270 nM for LAR, respectively. The initial rate of the PTPase-catalyzed hydrolysis of *p*-nitrophenol phosphate (pNPP) was measured by following the absorbance change at 405 nm. IC_{50} values were determined under a fixed pNPP concentration of 1 mM. All the assays were carried out in duplicate or triplicate, and the average results are presented. Inhibition constants (K_i) were derived from each IC_{50} based on competitive inhibition $K_i = IC_{50}K_m/(K_m + [\text{substrate}])$. For tight binding inhibitors, K_i is calculated from nonlinear fitting based on the tight binding equation

$$v = \frac{v_0}{2E_0} \times [\sqrt{(E_0 - I_0 - K_{app})^2 + 4E_0K_{app}} + (E_0 - I_0 - K_{app})]$$

$$K_i = K_{app}\left(\frac{K_m}{K_m + S}\right)$$

$$IC_{50} = K_i\left(\frac{K_m + S}{K_m}\right) + E_0/2$$

where v and v_0 are initial activity in the presence and the absence of inhibitors, E_0 is enzyme concentration, I_0 is inhibitor concentration, K_m is the Michaelis−Menten constant, and S is the substrate concentration. Data shown are representative of at least two independent determinations with a less than 2-fold difference between measurements.

PTPases used in the assays were recombinant human PTP1B (hPTP1B catalytic domain, residues 1−299, expressed and purified according to literature procedures[41]), recombinant human TCPTP (residues 1−299, expressed and purified in house), recombinant human CD45 (cytoplasmic domain, residues 484−1281, purchased from Biomol), and recombinant human LAR (soluble catalytic LAR-D1 domain, residues 1275−1613, purchased from Biomol).

5.3. X-ray Crystallographic Studies. Human recombinant PTP1B catalytic domain (residues 1−299) was prepared as described.[41] The complex solution was made by mixing an excess of inhibitor dissolved in DMSO with protein (protein:inhibitor = 1:1.5−3.0), incubating on ice for 1 h, and then filtering with 0.1 μm filters. Crystals of h-PTP1B−inhibitor complexes were obtained by vapor diffusion at 4 °C using 0.1 M HEPES (pH 7.0), 100−250 mM $MgCl_2$, 14−19% PEG 3350, 3 mM DTT, and 10−15 mg/mL protein. Typically, rodlike crystals appeared overnight and continued to grow to a maximum size of 0.2 mm × 0.2 mm × 0.7 mm within 10 days. The crystals belonged to space group $P3(1)21$, $a = b = 88$ Å, $c = 104$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. The crystals were cryoprotected by transferring into the crystallization solution 25% glycerol. Cryoprotected crystals were flash-cooled in liquid nitrogen prior to data collection.

Diffraction data of the h-PTP1B−inhibitor complex crystals were collected by either in-house conventional X-ray generator using Raxis-IV image detectors or using synchrotron radiation with CCD at ALS (Advanced Light Source in Lawrence National Laboratory at Berkeley, CA). All data were collected at 100 K and processed with DENZO/SCALPACK.

Table 8. Refinement Statistics of PTP1B−Inhibitor Complex Structures

compd	**16**	**19**	**23**	**32**
resolution (Å)	2.3	2.1	2.1	2.5
R-factor	.1997	.1927	.1942	.2089
R_{free}	.2315	.2287	.2255	.2451
rms[a] (b) (Å)	0.007	0.006	0.006	0.009
rms[b] (a) (deg)	1.34	1.25	1.41	1.47

[a] Root-mean-square bond deviation from standard. [b] Root-mean-square angle deviation from standard.

The structures were solved by molecular replacement with the available h-PTP1B catalytic domain structure model. Cycles of model rebuilding and refinement were carried out with CNS (version 1.1, Brunger, A.T., 2001, copyright Yale University), a system for X-ray crystallography; NMR; and QUANTA (Accelrys Inc.). Inhibitor was built into the difference density after the first cycle of refinement. Water molecules were assigned according to $F_o - F_c$ maps. The refinement statistics are summarized in Table 8.

5.4. Molecular Modeling. Analogs were initially docked into the active site of the PTP1b protein structure from the X-ray complex with compound **19**. As additional protein−inhibitor complex structures were determined, the binding site model was refined. Typically 1000 Monte Carlo cycles were carried out to dock each analog into the site, while also allowing selected protein residues to undergo constrained movement.[42] Figures 2−4 were generated using Benchware3D (Tripos, 2006, St. Louis, MO) and rendered with the Persistance of Vision Raytracer (POV-RAY) software (www.povray.org).

5.5. Pharmacokinetic and Metabolic Stability Studies. Pharmacokinetics of PTP1B inhibitors was determined in male C57/B6 mice (20−30 g, Taconic Farm, NY) after iv or ip administration. The compounds were prepared in DSM/PEG-200/saline solution for the iv administration or in 0.5% MC/2% TW/water as a suspension for the ip administration. Blood samples were collected over a period of 24 h, and plasma samples were harvested and stored at −80 °C until assay. The target tissues (liver and muscle) were also collected at selected time points. Tissues were homogenized in saline and the drug concentrations in the homogenates were analyzed. Quantization of PTP1B inhibitors in plasma and tissue homogenate was carried with a verified LC/MS/MS method.

In vitro metabolic half-life ($T_{1/2}$) and metabolic pathways were determined in liver microsomes from rats, mice, and humans using an NADPH-regenerating system consisting of $MgCl_2$ (10 mM), glucose-6-phosphate (3.6 mM), $NADP^+$ (1.3 mM) and glucose-6-phosphate dehydrogenase (0.4 units/mL) in sodium phosphate buffer (0.1 M, pH 7.4), UDPGA (4 mM), and substrate (1 mM for metabolic stability and 10 mM for metabolite profiling). Incubations were initiated by the addition of the NADPH-generating system and conducted for up to 30 min in a shaker−water bath at 37 °C. For the determination of in vitro metabolic half-life, aliquots of the incubation mixture were removed at 0, 10, 20, and 30 min and added to acetonitrile containing the appropriate internal standard. Following centrifugation and evaporation of the supernatant liquid, the samples were reconstituted in 20% methanol in water for analysis by LC/MS.

5.6. Studies of Compound Active Uptake into Hepatocytes. Rat primary hepatocytes (from a pool of seven or more livers) plated as a monolayer on 6-well collagen-coated plates were obtained from In Vitro Technologies, Inc. (Baltimore, MD). Incubations were based on a modification of previous methods (Kouzuki, H.; Suzuki, H.; Ito, K.; Ohashi, R.; Sugiyama, Y. Contribution of sodium taurocholate co-transporting polypeptide to the uptake of its possible substrates into rat hepatocytes. *J. Pharmacol. Exp. Ther.* **1998**, *286*, 1043−1050). Cells were washed three times with 2 mL of Krebs−Heinsleit buffer (KH; 142 mM NaCl, 23.8 mM Na_2CO_3, 4.83 mM KCl, 0.96 mM KH_2PO_4, 1.2 mM $MgSO_4$, 12.5 mM HEPES, 5 mM glucose, and 1.53 mM $CaCl_2$, pH 7.3) and preincubated for 5 min at 37 °C. Viability was assessed by microscopic examination. Fresh KH (1 mL) prewarmed to 37 °C containing compound **32** (10 μM final concentration) with and without 100 μM verapamil in DMSO/

MeOH (0.01/0.09% v/v) was added to each well and incubated at 37 °C in 5% CO_2 atmosphere for 5 min and terminated with the addition of 2 mL of ice-cold KH. Cells were washed three times with ice-cold KH, scraped, and homogenized with 200 μL of incubation media. Samples were extracted with acetonitrile containing internal standard, vortexed, and centrifuged at 21 000 g for 8 min at 4 °C. The supernatant was analyzed by LC/MS/MS with concentrations of compound **32** quantitated on the basis of interpolation from a six-point standard curve. Activity in the presence of verapamil, normalized for protein content (Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254), was reported as a percentage of activity in the absence of verapamil. Uptake of 10 μM fexofenadine was used as a positive control for OATP uptake.

Acknowledgment. The authors would like to acknowledge Nelson Huang, Ning Pan, and Walter Massefski for their role in the characterization of the small molecules published herein. The authors would also like to acknowledge the contributions to our PTP1B project by Rajeev Hotchandani, Michael J. Smith, Michelle Markus, Lydia Mosyak, Desiree Tsao, William S. Somers, and George Vlasuk.

Supporting Information Available: A table of HPLC purity. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Asante-Appiah, E.; Kennedy, B. P. Protein tyrosine phosphatases: The quest for negative regulators of insulin action. *Am. J. Physiol.* **2003**, *284*, E663−E670.

(2) Cook, W. S.; Unger, R. H. Protein tyrosine phosphatase 1B: A potential leptin resistance factor of obesity. *Dev. Cell* **2002**, *2*, 385−387.

(3) Byon, J. C. H.; Kusari, A. B.; Kusari, J., Protein-tyrosine phosphatase-1B acts as a negative regulator of insulin signal transduction. *Mol. Cell. Biochem.* **1998**, *182*, 101−108.

(4) Cheng, A.; Tremblay, M. L. Insulin receptor PTP: PTP1B. *Handb. Cell Signal.* **2004**, *1*, 729−732.

(5) Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* **1999**, *283*, 1544−1548.

(6) Klaman, L. D.; Boss, O.; Peroni, O. D.; Kim, J. K.; Martino, J. L.; Zabolotny, J. M.; Moghal, N.; Lubkin, M.; Kim, Y. B.; Sharpe, A. H.; Stricker-Krongrad, A.; Shulman, G. I.; Neel, B. G.; Kahn, B. B. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol. Cell. Biol.* **2000**, *20*, 5479−5489.

(7) Zinker, B. A.; Rondinone, C. M.; Trevillyan, J. M.; Gum, R. J.; Clampit, J. E.; Waring, J. F.; Xie, N.; Wilcox, D.; Jacobson, P.; Frost, L.; Kroeger, P. E.; Reilly, R. M.; Koterski, S.; Opgenorth, T. J.; Ulrich, R. G.; Crosby, S.; Butler, M.; Murray, S. F.; McKay, R. A.; Bhanot, S.; Monia, B. P.; Jirousek, M. R. PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11357−11362.

(8) Gum, R. J.; Gaede, L. L.; Koterski, S. L.; Heindel, M.; Clampit, J. E.; Zinker, B. A.; Trevillyan, J. M.; Ulrich, R. G.; Jirousek, M. R.; Rondinone, C. M. Reduction of protein tyrosine phosphatase 1B increases insulin-dependent signaling in ob/ob mice. *Diabetes* **2003**, *52*, 21−28.

(9) Hansen, S. K.; Cancilla, M. T.; Shiau, T. P.; Kung, J.; Chen, T.; Erlanson, D. A. Allosteric inhibition of PTP1B activity by selective modification of a non-active site cysteine residue. *Biochemistry* **2005**, *44*, 7704−7712.

(10) Zhang, Z.-Y.; Lee, S.-Y. PTP1B inhibitors as potential therapeutics in the treatment of type 2 diabetes and obesity. *Exp. Opin. Invest. Drug.* **2003**, *12*, 223−233.

(11) Tonks, N. K., PTP1B: From the sidelines to the front lines! *FEBS Lett.* **2003**, *546*, 140−148.

(12) Andersen, H. S.; Olsen, O. H.; Iversen, L. F.; Sorensen, A. L. P.; Mortensen, S. B.; Christensen, M. S.; Branner, S.; Hansen, T. K.; Lau, J. F.; Jeppesen, L.; Moran, E. J.; Su, J.; Bakir, F.; Judge, L.; Shahbaz, M.; Collins, T.; Vo, T.; Newman, M. J.; Ripka, W. C.; Moller, N. P. H. Discovery and SAR of a novel selective and orally bioavailable nonpeptide classical competitive inhibitor class of protein-tyrosine phosphatase 1B. *J. Med. Chem.* **2002**, *45*, 4443−4459.

(13) Dufresne, C.; Roy, P.; Wang, Z.; Asante-Appiah, E.; Cromlish, W.; Boie, Y.; Forghani, F.; Desmarais, S.; Wang, Q.; Skorey, K.; Waddleton, D.; Ramachandran, C.; Kennedy, B. P.; Xu, L.; Gordon, R.; Chan, C. C.; Leblanc, Y. The development of potent non-peptidic PTP-1B inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1039−1042.

(14) Larsen, S. D.; Stevens, F. C.; Lindberg, T. J.; Bodnar, P. M.; O'Sullivan, T. J.; Schostarez, H. J.; Palazuk, B. J.; Bleasdale, J. E. Modification of the N-terminus of peptidomimetic protein tyrosine phosphatase 1B (PTP1B) inhibitors: Identification of analogues with cellular activity. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 971−975.

(15) Lau, C. K.; Bayly, C. I.; Gauthier, J. Y.; Li, C. S.; Therien, M.; Asante-Appiah, E.; Cromlish, W.; Boie, Y.; Forghani, F.; Desmarais, S.; Wang, Q.; Skorey, K.; Waddleton, D.; Payette, P.; Ramachandran, C.; Kennedy, B. P.; Scapin, G. Structure based design of a series of potent and selective non peptidic PTP-1B inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1043−1048.

(16) Liu, G.; Xin, Z.; Liang, H.; Abad-Zapatero, C.; Hajduk, P. J.; Janowick, D. A.; Szczepankiewicz, B. G.; Pei, Z.; Hutchins, C. W.; Ballaron, S. J.; Stashko, M. A.; Lubben, T. H.; Berg, C. E.; Rondinone, C. M.; Trevillyan, J. M.; Jirousek, M. R. Selective protein tyrosine phosphatase 1B inhibitors: Targeting the second phosphotyrosine binding site with non-carboxylic acid-containing ligands. *J. Med. Chem.* **2003**, *46*, 3437−3440.

(17) Zhao, H.; Liu, G.; Xin, Z.; Serby, M. D.; Pei, Z.; Szczepankiewicz, B. G.; Hajduk, P. J.; Abad-Zapatero, C.; Hutchins, C. W.; Lubben, T. H.; Ballaron, S. J.; Haasch, D. L.; Kaszubska, W.; Rondinone, C. M.; Trevillyan, J. M.; Jirousek, M. R. Isoxazole carboxylic acids as protein tyrosine phosphatase 1B (PTP1B) inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5543−5546.

(18) Shen, K.; Keng, Y. F.; Wu, L.; Guo, X. L.; Lawrence, D. S.; Zhang, Z. Y. Acquisition of a specific and potent PTP1B inhibitor from a novel combinatorial library and screening procedure. *J. Biol. Chem.* **2001**, *276*, 47311−9.

(19) Johnson, T. O.; Ermolieff, J.; Jirousek, M. R. Protein tyrosine phosphatase 1B inhibitors for diabetes. *Nat. Rev. Drug Discovery* **2002**, *1*, 696−709.

(20) Liu, G. Protein tyrosine phosphatase 1B inhibition: Opportunities and challenges. *Curr. Med. Chem.* **2003**, *10*, 407−1421.

(21) Tobin, J. F.; Tam, S. Recent advances in the development of small molecule inhibitors of PTP1B for the treatment of insulin resistance and type 2 diabetes. *Curr. Opin. Drug Discovery Dev.* **2002**, *5*, 500−512.

(22) Andersen, J. N.; Tonks, N. K. Protein tyrosine phosphatase-based therapeutics: Lessons from PTP1B. *Top. Curr. Genet.* **2004**, *5*, 201−230.

(23) Montalibet, J.; Kennedy, B. P. Therapeutic strategies for targeting PTP1B in diabetes. *Drug Discovery Today: Ther. Strategies* **2005**, *2*, 129−135.

(24) Moretto, A. F.; Kirincich, S. J.; Xu, W. X.; Smith, M. J.; Wan, Z.-K.; Wilson, D. P.; Follows, B. C.; Binnun, E.; Joseph-McCarthy, D.; Foreman, K.; Erbe, D. V.; Zhang, Y. L.; Tam, S. K.; Tam, S. Y.; Lee, J. Bicyclic and tricyclic thiophenes as protein tyrosine phosphatase 1B inhibitors. *Bioorg. Med. Chem.* **2006**, *14*, 2162−2177.

(25) Wan, Z.-K.; Lee, J.; Xu, W. X.; Erbe, D. V.; Joseph-McCarthy, D.; Follows, B. C.; Zhang, Y. L. Monocyclic thiophenes as protein tyrosine phosphatase 1B inhibtors: Capturing interactions with Asp48. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4941−4945.

(26) Iversen, L. F.; Andersen, H. S.; Moller, K. B.; Olsen, O. H.; Peters, G. H.; Branner, S.; Mortensen, S. B.; Hansen, T. K.; Lau, J.; Ge, Y.; Holsworth, D. D.; Newman, M. J.; Hundahl Moller, N. P. Steric hindrance as a basis for structure-based design of selective inhibitors of protein-tyrosine phosphatases. *Biochemistry* **2001**, *40*, 14812−20.

(27) Puius, Y. A.; Zhao, Y.; Sullivan, M.; Lawrence, D. S.; Almo, S. C.; Zhang, Z. Y. Identification of a second aryl phosphate-binding site in protein-tyrosine phosphatase 1B: A paradigm for inhibitor design. *Proceed. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13420−13425.

(28) Asante-Appiah, E.; Patel, S.; Dufresne, C.; Roy, P.; Wang, Q.; Patel, V.; Friesen, R. W.; Ramachandran, C.; Becker, J. W.; Leblanc, Y.; Kennedy, B. P.; Scapin, G. The structure of PTP-1B in complex with a peptide inhibitor reveals an alternative binding mode for bisphosphonates. *Biochemistry* **2002**, *41*, 9043−9051.

(29) Szczepankiewicz, B. G.; Liu, G.; Hajduk, P. J.; Abad-Zapatero, C.; Pei, Z.; Xin, Z.; Lubben, T. H.; Trevillyan, J. M.; Stashko, M. A.; Ballaron, S. J.; Liang, H.; Huang, F.; Hutchins, C. W.; Fesik, S. W.; Jirousek, M. R. Discovery of a potent, selective protein tyrosine phosphatase 1B inhibitor using a linked-fragment strategy. *J. Am. Chem. Soc.* **2003**, *125*, 4087−4096.

(30) Smith, M. B.; March, J. *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 5th ed.; Wiley: New York, 2000; 1824 pp.

(31) Baxter, E. W.; Reitz, A. B. Reductive aminations of carbonyl compounds with borohydride and borane reducing agents. *Org. React. (New York)* **2002**, *59*, 1−714.

(32) Abdel-Magid, A. F.; Maryanoff, C. A. Use of sodium triacetoxyborohydride in reductive amination of ketones and aldehydes. *ACS Symp. Ser.* **1996**, *641* (Reductions in Organic Synthesis), 201−216.

(33) Corral, C.; Lissavetzky, J. Reactions with 3-hydroxy-2-(methoxycarbonyl)thiophene; I. Synthesis of 3-thienyloxyacetic acid and its (nuclear) chloro and bromo derivatives. *Synthesis* **1984**, (*10*), 847−50.

(34) Pedersen, A. K.; Peters, G. u. H.; Moller, K. B.; Iversen, L. F.; Kastrup, J. S. Water-molecule network and active-site flexibility of apo protein tyrosine phosphatase 1B. *Acta Crystallogr. Sect. D: Biol. Crystallogr.* **2004**, *D60*, 1527−1534.

(35) Scapin, G.; Patel, S.; Patel, V.; Kennedy, B.; Asante-Appiah, E. The structure of apo protein-tyrosine phosphatase 1B C215S mutant: More than just an S → O change. *Protein Sci.* **2001**, *10*, 1596−1605.

(36) Iversen, L. F.; Moller, K. B.; Pedersen, A. K.; Peters, G. H.; Petersen, A. S.; Andersen, H. S.; Branner, S.; Mortensen, S. B.; Moller, N. P. H. Structure determination of T cell protein-tyrosine phosphatase. *J. Biol. Chem.* **2002**, *277*, 19982−19990.

(37) Cvetkovic, M.; Leake, B.; Fromm, M. F.; Wilkinson, G. R.; Kim, R. B. OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab. Dispos.* **1999**, *27*, 866−871.

(38) Shimizu, M.; Fuse, K.; Okudaira, K.; Nishigaki, R.; Maeda, K.; Kusuhara, H.; Sugiyama, Y. Contribution of OATP (organic anion-transporting polypeptide) family transporters to the hepatic uptake of fexofenadine in humans. *Drug Metab. Dispos.* **2005**, *33*, 1477−1481.

(39) Poirier, A.; Funk, C.; Lave, T.; Noe J. New strategies to address drug−drug interactions involving OATPs. *Curr. Opin. Drug Discovery Dev.* **2007**, *10*, 74−83.

(40) Corral, C.; Lissavetzky, J.; Manzanares, I. Effects of substituents on the chlorination of methyl 3-hydroxythiophene-2-carboxylate and on the reaction of the resulting chlorinated products with active hydrogen containing compounds. *J. Heterocycl. Chem.* **1990**, *27*, 315−319.

(41) Hoppe, E.; Berne, P.-F.; Stock, D.; Rasmussen, J. S.; Moeller, N. P. H.; Ullrich, A.; Huber, R. Expression, purification and crystallization of human phosphotyrosine phosphatase 1B. *Eur. J. Biochem.* **1994**, *223*, 1069−1077.

(42) McMartin, C.; Bohacek, R. S. QXP: Powerful, rapid computer algorithms for structure-based drug design. *J. Comput.-Aided Mol. Design* **1997**, *11*, 333−344.

JM0702478