Discovery and SAR of a Novel Selective and Orally Bioavailable Nonpeptide Classical Competitive Inhibitor Class of Protein-Tyrosine Phosphatase 1B

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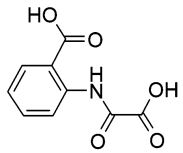
Reversible phosphorylation and dephosphorylation of key proteins on tyrosine residues are important parts of intracellular signaling triggered by hormones and other agents. Recent knock-out studies in mice have identified PTP1B as a potential target for the treatment of diabetes and obesity. As a consequence, a number of academic and industrial groups are aggressively pursuing the development of selective PTP1B inhibitors. In addition, other protein−tyrosine phosphatases (PTPs) appear to be critically involved in major diseases such as cancer and autoimmunity. Given the diversity of PTPs and their potential as drug targets in different diseases, we have taken a broad approach to develop active site-directed selective inhibitors of specific members of this family of enzymes. Using a high throughput screening, we have previously identified 2-(oxalylamino)benzoic acid **3a** as a relatively weak but classical competitive inhibitor of several PTPs.[4] On the basis of our early studies, indicating that **3a** might be used as a starting point for the synthesis of selective PTP inhibitors, we now present our efforts in expansion of this concept and provide here a number of new chemical scaffolds for the development of inhibitors of different members of the PTP family. Although the core structure of these inhibitors is charged, good oral bioavailability has been observed in rat for some compounds. Furthermore, we have observed enhancement of 2-deoxy-glucose accumulation in C2C12 cells with prodrug analogues.

Introduction

Reversible tyrosine phosphorylation plays a pivotal role in most cellular signaling processes. Protein tyrosine kinases (PTKs) phosphorylate cellular substrates on tyrosine residues, and protein−tyrosine phosphatases (PTPs) remove phosphate from tyrosine-phosphorylated proteins (for reviews, see refs 1 and 2). It is generally believed that low molecular weight selective PTP inhibitors could potentially be used for the treatment of a variety of diseases such as diabetes, autoimmunity, and cancer. Recently, it was shown that mice lacking functional PTP1B exhibit increased insulin sensitivity and resistance to diet-induced obesity, thus pointing to this enzyme as an attractive drug target in diabetes and obesity.[3]

We have recently identified 2-(oxalylamino)benzoic acid **3a** (OBA, Figure 1) as a general inhibitor of PTPs.[4] Importantly, OBA seems to be one of the most potent "minimal unit" phenyl phosphate mimetics identified so far.[5] X-ray protein crystallography of PTP1B cocrystallized with OBA revealed that it binds to the highly conserved phosphate binding loop (the PTP loop), thus mimicking part of the binding pattern of the natural substrate.[6,7] In addition, OBA exhibits a novel binding pattern, interacting with other residues (e.g., Lys120) surrounding the active site, which are not directly involved in substrate binding. Recently, Bleasdale et al. demonstrated a similar binding pattern for 2-carboxymethoxybenzoic acid-based inhibitors, which also show interaction to Lys120.[8] Because of OBA's low molecular weight and its enzyme kinetic behavior as a classical, time-independent competitive inhibitor, this compound was used as a starting point for structure-based lead optimization to develop selective small molecule inhibitors of PTP1B.



3a (OBA)

Figure 1.

To obtain selectivity for PTP1B against the expected ∼40 different human PTPs[9] is a key issue. With the aim of identifying unique combinations of amino acid

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residues that could be used in a structure-based approach to design selective inhibitors, we have recently performed a detailed comparison of all published PTPs.[10] The analysis was based on both primary sequence alignments of PTP domains and low resolution homology modeling (Cα regiovariation score analysis). Because OBA was selected as our starting point, we were particularly interested in identifying combinations of residues in the vicinity of the active site, i.e., at a distance that would allow simultaneous binding to the active site and these residues. Several potential selectivity determining areas were identified including a region defined by residues 47, 48, 258, and 259. Asp48 is well-defined, as judged by B factors in the published PTP1B structures,[11,12] and it is believed to play an important role in positioning substrates relative to the active site.[13]

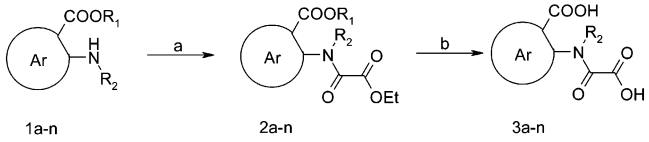
Residue 48 seemed a particularly attractive binding element for the development of selective PTP1B ligands since this is an aspartic acid in PTP1B and an asparagine in many other PTPs. Before we entered into a major optimization effort, we first tested our hypothesis with a relatively simple derivative as described recently.[14] This initial study provided structural and enzyme kinetic evidence for the notion that a correctly positioned basic nitrogen in the core structure of this low molecular weight inhibitor could form a salt bridge with Asp48 in PTP1B. In other PTPs with an asparagine in the equivalent position, the basic nitrogen would cause repulsion and hence lead to selectivity against these enzymes. In the present study, we now describe a comprehensive analysis of this novel general PTP inhibitor scaffold (**3a**), a detailed structure−activity relationship (SAR), and its development into a moderately potent, selective, and orally available inhibitor of PTP1B.

Chemistry

First, we optimized for potency in the hydrophobic part of the tyrosine phosphate binding pocket by introducing a number of hydrophobic substituents at various positions at the aromatic ring. Next, we looked for phenyl replacement with pyridine, thiophenes, naphthalene, and indoles using the general synthetic procedure as outlined in Table 1. In brief, aromatic ortho-amino acids or esters **1a**−**n** were treated with ethyl oxalyl chloride in tetrahydrofuran (THF) affording mono- or diesters of general structure **2a**−**n**. Basic hydrolysis of the ester groups gave after treatment with aqueous HCl the desired oxalylamino anthranilic acid **3a** and analogues **3b**−**n**.

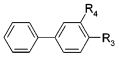
The naphthalene and indole derivatives **3l**,**m** were found to be the most potent phenyl replacements (see Table 1). However, substituted derivatives thereof—in particular of naphthalene—are difficult to synthesize. Because the thiophene compound **3g** and OBA were almost equipotent, we therefore decided to use **3g** for further optimization. Molecular modeling indicated that hydrophobic substituents at the 5-position of the thiophene nucleus should be favored. Utilizing the method described by Hartmann and Liebscher[15] for the synthesis of 5-aryl-3-amino-2-alkoxycarbonyl-thiophenes as shown in Scheme 1, a series of 5-aryl-thiophenes **8a**−**o** were synthesized starting from acetophenones **4a**−**o**, which were treated with phosphorus oxychloride in dimethylformamide (DMF) followed by hydroxylamine affording β-chlorocinnamonitriles **5a**−**o** in moderate yields. β-Chlorocinnamonitriles **5a**−**o** underwent cyclization yielding 5-aryl-3-amino-2-alkoxycarbonyl-thiophenes **6a**−**o** when reacted with α-mercaptoacetic

Table 1. Inhibitory Effect of **3a**−**n** on PTP1B, PTPα, and LAR[a]



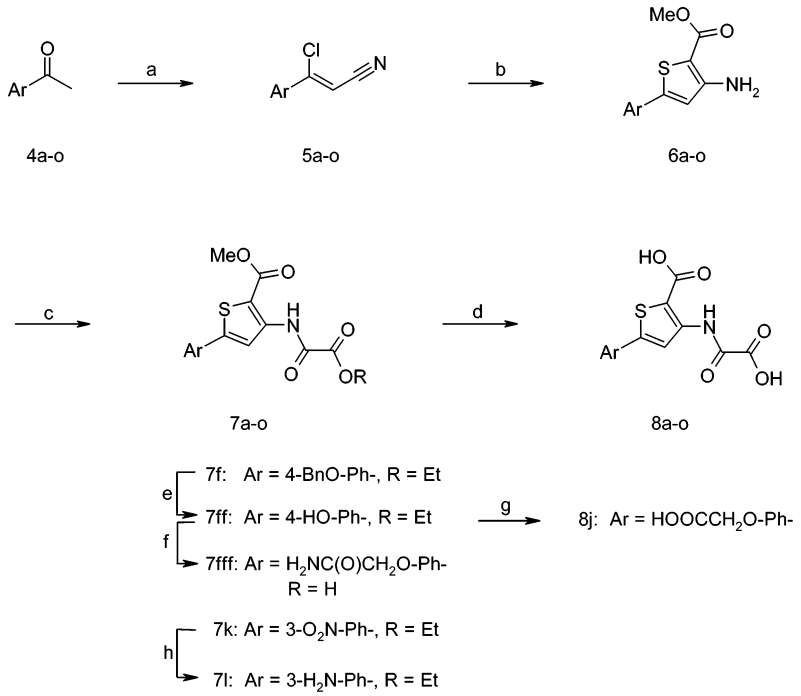
1a-n 2a-n 3a-n

R_1 = H, Me, Et

Compd	Ar[c]	R_2	K_i (μM)[b] PTP1B	PTPα	LAR
3a[4]		H	23	870	2000
3b		H	1700	2000	2000
3c		H	1100	2000	2000
3d		Me	1100	2000	2000
3e[14]		H	108	1700	2000
3f		H	75	ND	ND
3g[14]		H	37	2000	2000
3h		H	2000	2000	2000
3i[4]		H	14	89	420
3j		H	14	280	700
3k		H	160	1000	2000
3l[4]		H	9.9	190	68
3m[4]		H	8	180	450
3n[4]		H	18	800	550

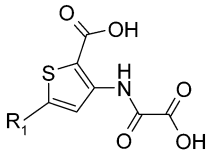
[a] Reagents: (a) EtOOCCOCl, TEA, THF, 0 °C. (b) NaOH, H_2O, EtOH and then HCl. [b] K_i values are measured at pH 5.5. [c] The oxalylamide is attached at the R_3 position and the carboxy group is attached at the R_4 position.

Scheme 1[a]



[a] Reagents: (a) DMF, $POCl_3$, $H_2N{-}OH{\cdot}HCl$, 25-50 °C. (b) $HSCH_2COOMe$, NaOMe/MeOH. (c) EtOOCOCl, THF. (d) NaOH, EtOH, H_2O, and then HCl. (e) 33% HBr/AcOH, $CH_3(CH_2)_5P((CH_2)_3CH_3)_3^+Br^-$. (f) (i) $BrCH_2CONH_2$, K_2CO_3, DMF, 50 °C; (ii) aqueous HCl. (g) 1 N NaOH, MeOH. (h) 10% Pd/C, H_2, EtOH/THF.

Table 2. Inhibitory Effect of **3g** and **8a**−**o** on PTP1B, PTPα, and LAR



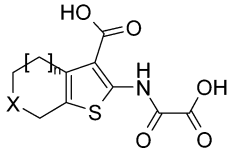
compd	R_1	K_i (μM)[a] PTP1B	PTPα	LAR
3g	H	37	2000	2000
8a	Ph	13	250	90
8b	3-thienyl	11	200	90
8c	4-(*i*-Bu)-Ph	13	100	50
8d	4-F-Ph	8	210	110
8e	4-Cl-Ph	10	100	160
8f	4-HO-Ph	4.5	210	ND
8g	4-MeO-Ph	9	150	ND
8h	4-PhO-Ph	26	93	ND
8i	4-BnO-Ph	16	65	ND
8j	4-($HOOCCH_2$-O)-Ph	2.5	200	50
8k	3-NO_2-Ph	4.7	700	165
8l	3-H_2N-Ph	6.1	250	113
8m	3-MeO-Ph	12	530	ND
8n	3,4-MeO-Ph	25	980	ND
8o	3,5-MeO-Ph	8	1789	ND

[a] K_i values are measured at pH 5.5 using pNPP as substrate.

acid methyl ester in the presence of a base. Treatment of compounds **6a**−**o** with ethyl oxalyl chloride in THF followed by basic hydrolysis of the two ester groups gave after acidic workup the desired 5-aryl-thiophenes **8a**−**o** as shown in Table 2. Compound **8j** was obtained in a slightly different way via **7f**,**ff** as shown in Scheme 1. Although relatively potent compounds with K_i values in the low micromolar range at pH 5.5 (PTP1B) were obtained in this 5-aryl-thiophene series, the acidic reaction conditions used in the first step prevented the introduction of acid sensitive substituents, thus limiting the scope of this approach. In addition, a moderate yield in this step prompted us to use 2-oxalylaminothiophene **3e** as a starting point.

On the basis of FlexX[16] docking and ease of synthesis, a number of fused 2-aminothiophene ring systems were proposed as candidates for positioning heteroatoms, in particular a basic nitrogen, in a distance sufficiently close to allow hydrogen bonding or salt bridge formation to Asp48. Fused 2-amino-thiophenes (Table 3) of general structure **12a**−**k** were prepared as shown in Scheme 2. Appropriate ketones **9a**−**k** were condensed using Gewald's 2-aminothiophene reaction[17] with ethyl/*tert*-butyl cyanoacetate, sulfur, and morpholin affording fused 2-amino-thiophenes **10a**−**k** followed by acylation with ethyl oxalyl chloride or imidazol-1-yl-oxo-acetic acid *tert*-butyl ester[18] to give the diesters **11a**−**k**, which were deprotected with aqueous NaOH or trifluoroacetic acid (TFA)/DCM affording **12a**−**k**. In the case of X being nitrogen, we have developed different synthetic routes allowing introduction of substituents at the nitrogen atom. First, we synthesized the unsubstituted thieno-[2,3-c]pyridine **12h** (R_1 = H) using N-Boc protected 4-piperidone **13b** and *tert*-butyl cyanoacetate leading to **19b** in high yield as shown in Scheme 3. The oxalyl side chain was introduced via its *tert*-butyl ester, which allowed simultaneous deprotection of **20b** in the final step using 25% TFA in dichloromethane. In a second round of optimization, FlexX dockings[16] indicated that a lipophilic cleft (defined by Met258, Gly259, Gln262, Arg254, Arg24, and Asp48) in PTP1B could be addressed by introduction of substituents at the basic nitrogen in the tetrahydro-thieno[2,3-c]pyridine ring. In most other PTPs, bulky amino acid side chains in position 259 prevent binding of inhibitors when addressing this lipophilic area of PTP1B.[10,19,20] We have used three different routes, which allowed introduction

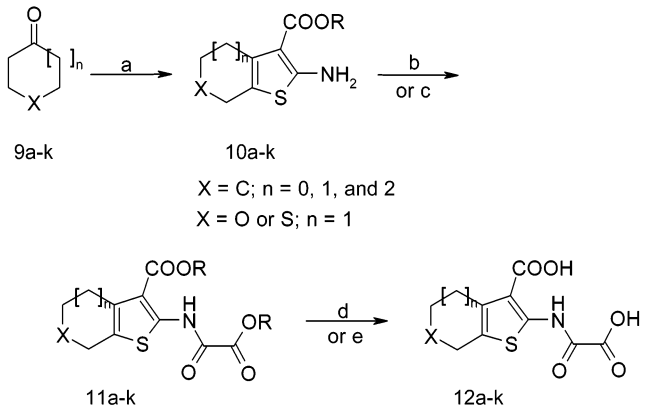
Table 3. Inhibitory Effect of Fused 2-Aminothiophenes **12a**−**k** on PTP1B, PTPα, and LAR



Compd	X	n	K_i (μM)[a] PTP1B	PTPα	LAR
12a	CH_2	0	12	340	900
12b	CH_2	1	8.1	290	410
12c	CH_2	2	12	320	400
12d	O	1	14	170	360
12e	S	1	8.3	100	ND
12f	SO	1	5.1	363	ND
12g	SO_2	1	6.1	322	ND
12i	C=O	1	1.3	52	ND
12j	CH-OH	1	2.0	215	ND
12k		1	2.2	150	ND

[a] K_i values are measured at pH 5.5 using pNPP as substrate; *, attachment point.

Scheme 2



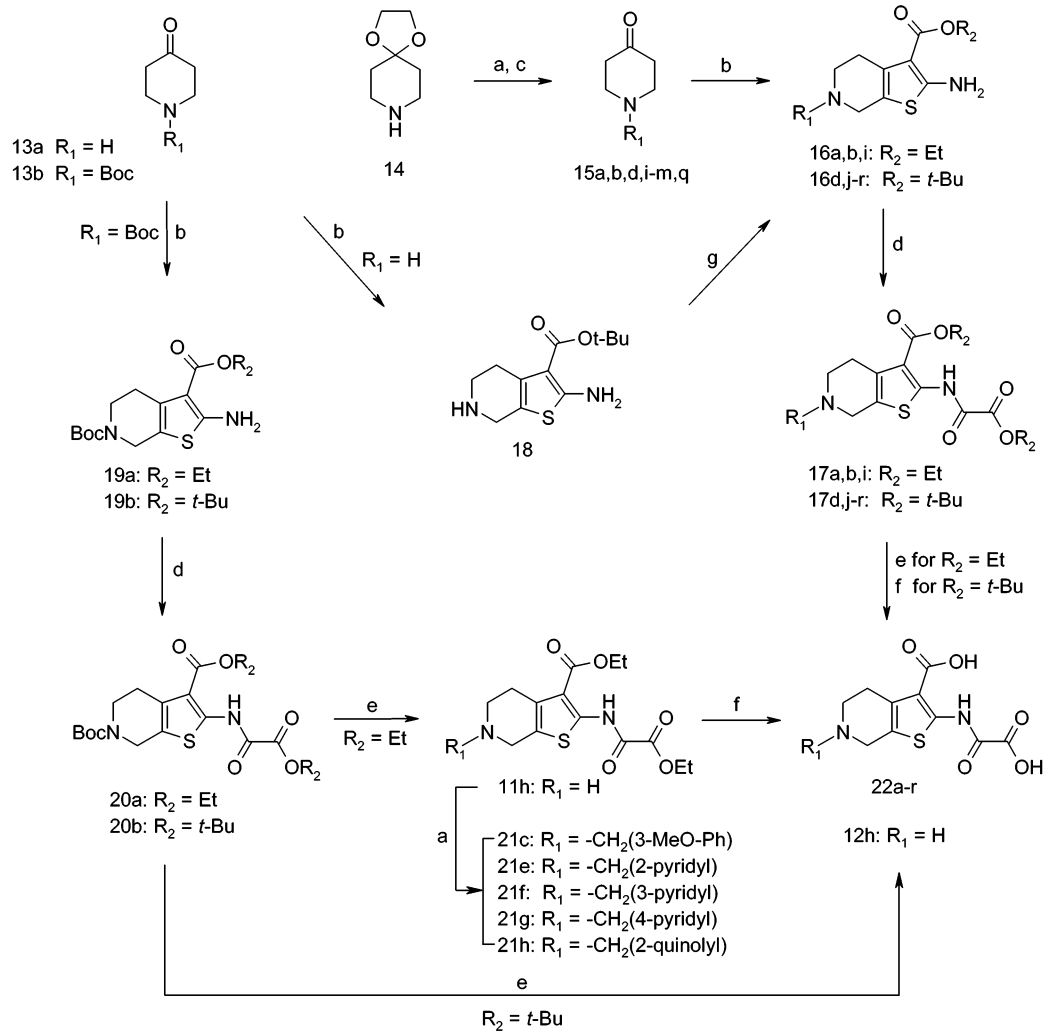
of a variety of lipophilic side chains. Commercially available N-alkylated and N-acylated 4-piperidones **15a**,**b**,**i** were reacted as shown in Scheme 3 affording N-alkylated and N-acylated thieno[2,3-c]pyridines **22a**,**b**,**i**, respectively. The late intermediate **11h** (R_1 = H) readily available from **13b** was next used in a short convergent synthesis of additional N-alkylated thieno[2,3-c]pyridines. Reacting compound **13b** with aryl−alkyl halides using K_2CO_3 in acetone or methyl *iso*-butyl ketone (MIBK) as shown in Scheme 3 produced N-alkylated thieno[2,3-c]pyridines **21c** and **22e**−**h** in moderate to low yield—in part due to instability of the ethyl oxalyl ester. Deprotection with aqueous NaOH followed by precipitation of the inner salt with aqueous HCl afforded compounds **22c**,**e**−**h**. Because of the observed instability, a more robust synthesis was needed. Using a less convergent approach, also shown in Scheme 3, produced more stable intermediates, providing us with a robust synthesis of further N-alkylated thieno[2,3-c]pyridines. Dioxolane **14** was N-alkylated using either alkyl-halides or alkyl-mesylates and K_2CO_3 in MIBK followed by deketalization, which afforded N-alkylated thieno[2,3-c]pyridines of general structure **16d**,**j**−**m**,**q**. The oxalyl side chain was introduced under very mild conditions using imidazol-1-yl-oxo-acetic acid *tert*-butyl ester[18] in THF producing the desired compounds **22d**,**j**−**m**,**q** after TFA cleavage. Recently, a convenient synthesis was published describing direct alkylation of amines with alcohols[21] with the aid of (cyanomethyl)-trimethylphosphonium iodine. Utilizing this procedure in our optimization process, the secondary amine **18** was selectively alkylated at the secondary nitrogen followed by oxalation with imidazol-1-yl-oxo-acetic acid *tert*-butyl ester affording di-*tert*-butyl esters of formula **17b**,**d**,**n**−**p**,**r**. Deprotection was accomplished using TFA in DCM yielding **22b**,**d**,**n**−**p**,**r**.

Results and Discussion

All new compounds—which showed satisfactory combustion analysis—were tested against a soluble form of recombinant human PTP1B (amino acid residues 1−321), using *p*-nitrophenyl phosphate (pNPP) as substrate as previously described.[4,14] To verify specificity, selected compounds were also tested against other recombinant PTPs including TC-PTP.

Minimal Units/Core Structures. The key structural features of OBA are the two carboxy groups bound, directly and through a carbonylamino group, to an aromatic ring (Figure 1, OBA). We have previously reported the synthesis and enzyme kinetic properties of a few derivatives of OBA.[4] In the following, a detailed structure−activity analysis will be presented. As described above, OBA shares several interaction points with the natural substrate, phosphotyrosine (pTyr), within the PTP loop. However, superimposition of OBA and pTyr in complex with PTP1B also shows that the aromatic cores are only partly overlapping. Thus, we reasoned that improving the potency of OBA could be achieved by increasing the van der Waals contacts between the ligand and the hydrophobic part of the phenyl binding pocket. To address this, we introduced a number of hydrophobic substituents at various positions at the aromatic ring. Methyl substituents at the 2- and 6-position and at the oxalylamide nitrogen atom lead to inactive compounds **3b**−**d**, most likely due to steric hindrance and to change of the optimal binding conformation of the ligand, whereas the 5-position was able to accommodate even large hydrophobic groups such as iodo **3i** and phenyl **3j** resulting in a small increase in potency as shown in Table 1. As previously reported,[4,14] the phenyl ring in OBA could be replaced with the thiophene **3e**,**g**, naphthalene **3l**, and indole **3m**,**n** and here, we have further expanded the list with thiophene **3f**. In contrast, replacement of the phenyl ring with the more polar pyridine **3k** resulted in a 7-fold decrease in potency indicating that this pyridine regioisomer is less favorable. Assuming similar binding modes for compound **3k** and OBA, we speculate that the nitrogen lone pair in **3k** causes a disruption of the van der Waals interaction with Ile219. In agreement with the above results—indicating a rather limited space

Scheme 3[a]



[a] Reagents: (a) R_1-X (X = Cl, Br, or OMs), K_2CO_3, KI, acetone or MIBK, heat. (b) $NCCH_2COOR_2$ (R_2 = Et or *t*-Bu), sulfur, EtOH, morpholine, 50 °C. (c) HCl, dioxan, heat. (d) EtOOCCOCl, THF, TEA, *t*-BuOOCCOimidazol-1-yl, THF. (e) 25% TFA/CH_2Cl_2. (f) NaOH, EtOH, H_2O and then aqueous HCl. (g) R_1-OH, $(Me)_3P^+CH_2CN$, I^-, Pr−CN, DIPEA.

in the phenyl binding pocket—the thiophene-based compounds **3e**−**g** showed an increase in potency going from 2-(oxalylamino)thiophene-3-carboxylic acid (**3e**) toward 3-(oxalylamino)thiophene-2-carboxylic acid (**3g**), the latter being almost equipotent to OBA (Table 1). This difference in potency for the three regioisomers can in part be explained by the assumptions that the bulky sulfur atom in compound **3e** is positioned too close to the side chains of Val49 and Ile219 thereby introducing steric hindrance as compared to compound **3f**,**g**, where the sulfur atom has been moved away. Also, in the thiophene series, introduction of a methyl substituent ortho to the oxalylamide side chain in **3g** gave rise to an inactive compound (**3h**), again supporting the notion that the phenyl binding pocket is quite narrow.

Nonfused Amino-Thiophenes. We have previously shown that enhanced potency can be obtained by introduction of an additional ring in the core structure, as exemplified by **3l**−**n**. X-ray crystallography demonstrated that the observed increase in potency is due to extensive hydrophobic interactions with the side chains of Tyr46, Phe182, Ala217, and Ile219.[4] However, because it is difficult to synthesize mono- and disubstituted derivatives of compound **3l**, a replacement for the phenyl/naphthyl moiety was necessary. Although **3i**,**j** were attractive molecules from an optimization point of view, molecular modeling indicated that the thiophene-based scaffold **3g** was offering a more attractive position—the 5-position—for the introduction of substituents. To mimic the favorable van der Waals interactions previously seen with compounds **3l**[4] and **3i**,[14] a number of phenyl, substituted phenyl, and thienyl-based compounds **8a**−**e** (Table 2) were synthesized. When compared with compound **3g**, compounds **8d**,**e** showed up to a 4-fold increase in potency for the fluoro and chloro, thus supporting the above hypothesis. Interestingly, these compounds showed the same inhibitor profile as seen for compound **3l** when compared with PTPα and LAR.

To provide information for further optimization of this series of compounds, PTP1B was cocrystallized with compound **8e** (Figure 2). All interactions involving the oxalylamide and the *ortho*-carboxylic acid groups with the PTP loop and Lys120, respectively, were found to be the same as those previously described for compound **12h**.[14] As in PTP1B complexed with a peptide substrate,[22] we observed a water molecule similarly positioned and trapped under the WPD loop. In our case,

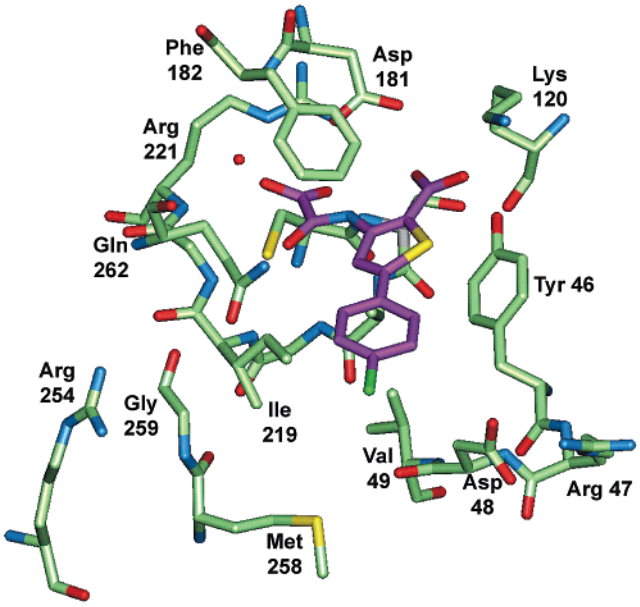


Figure 2. X-ray structure (2.00 Å resolution) of PTP1B cocrystallized with compound **8e**. The binding mode of compound **8e** in the active site pocket of PTP1B is shown. Atoms are colored according to atom type (carbon in light green and violet for compound **8e**, oxygen in red, sulfur in yellow, nitrogen in blue, and chloro in green).

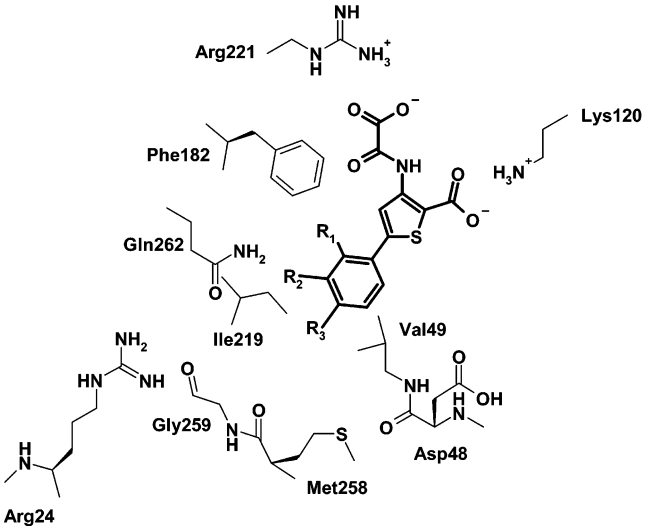


Figure 3. The nonfused 3-amino-5-aryl thiophene scaffold offers a novel and unique possibility for introduction of substituents at different positions.

this water molecule forms a hydrogen bond with the oxalyl amide carbonyl. As anticipated from modeling studies, the phenyl ring forms a set of van der Waals interactions with Ile219 and Val49. Ligand binding forces the side chain of Asp48 into a novel rotamer conformation, which allows the formation of a novel set of van der Waals contacts between the α-carbon atom of Asp48 and the phenyl ring. This rotamer conformation (rota 1) seems to be less favored based on its occurrence in only four of the 11 published X-ray structures. However, similar to the structure presented here (Figure 2), the rota 1 position was required to accommodate ligands. From a structure-based design point of view, this rotamer position is highly desirable, since it allows addressing the cleft surrounded by Arg24, Arg254, Met258, Gln259, and Asp48 in PTP1B. In our series of X-ray structures of PTP1B co-crystallized with a number of different oxalylamide-based PTP1B inhibitors, this part of PTP1B is always filled with water molecules forming a diverse set of interactions with the amino acid side chains (not shown). Substituting one or more of these water molecules with a small polar group such as hydroxy should give rise to an increase in potency for PTP1B and at the same time serve as a handle for introduction of further substituents. In agreement with this notion, substituting the chloro atom with a hydroxy group affording **8f** gave an almost 3-fold increase in potency when compared with **8a**. Introduction of the more flexible polar acetic acid residue affording compound **8j** gave a further 2-fold increase in potency. Although the above-mentioned cleft is partly hydrophobic (Met258 and Gln259), introduction of lipophilic substituents such as methyl, phenyl, and benzyl at the hydroxy group leading to **8g**−**i** in all cases resulted in a marked decrease in potency.

In all of our PTP1B X-ray structures, Gln262 is found in a locked conformation hydrogen bonding to the nitrogen backbone atom of Gly259, which makes this side chain residue attractive from a structure-based design point of view. On the basis of the above X-ray structure of compound **8e**, the meta position of the phenyl ring points toward this Gly259. Introduction of substituents capable of hydrogen bonding produced compounds **8k**−**o**. Of interest, compound **8k** showed an almost 3-fold increase in potency for the nitro group and a little less for the corresponding amino group, compound **8l**. In contrast and in agreement with the above X-ray structure, the methoxy substituents in compound **8m**−**o** are not well-accommodated. Of note, in particular, compound **8o** shows a 7-fold increase in selectivity toward PTPα when compared with **8a**, supporting our previous reports that selectivity can be obtained in this area of the enzymes.[19,20] In conclusion, we believe that the described nonfused 3-amino-5-aryl-thiophene scaffold offers a novel and unique possibility for further optimization for potency and selectivity particularly around Gly259 and Met258 and around Ile219 and Gln262 as shown in Figure 3.

In the 5-aryl-thiophene series, the acidic reaction conditions used in the first step prevent introduction of acid sensitive substituents. Therefore, to broaden our repertoire of synthetic scaffolds, we next investigated 2-oxalylamino-thiophene **3e**.

Fused Amino-Thiophenes. We and others have previously shown that fused ring systems markedly increase the overall potency against PTP1B due to additional van der Waals interactions to the phenyl phosphate binding pocket.[4,23,24] Using modeling, FlexX[16] docking, and ease of synthesis as filters, a number of aryl- and cycloalkyl-fused 2-amino-thiophene ring systems were proposed as candidates for regioselective positioning of small substituents, in particular heteroatoms.

By way of example, a variety of fused 2-aminothiophenes were synthesized (Table 3). Among the cycloalkyl-fused analogues **12a**−**c**, compound **12b** showed the same potency against PTP1B as compound **3l**, and it is 3-fold more potent than OBA when tested at pH 5.5 (the pH optimum for PTP1B and the rat analogue PTP1 using *p*NPP as substrate).[25] Importantly, all three

Table 4. Amino Acid Residues at Positions in the Vicinity of the Active Site (Three Letter Code, PTP1B Numbering)

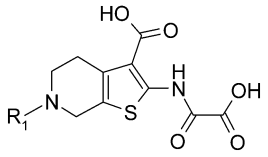
residue	PTP1B	SHP-1	PTP-L1	PTPD2	PTPH1	STEP	IA-2	PTPβ	PTPα	PTPϵ	LAR	PTPμ	CD45	PTPγ
47	Arg	Lys	Lys	Arg	Lys	Lys	Pro	Asn	Val	Pro	Ala	Gly	Val	Ile
48	Asp	Asn	Asn	Glu	Asp	Thr	Asp	Asn	Asn	Asn	Asn	Asn	Asp	Asn
258	Met	Ser	His	Met	Ala	Gly	Pro	Val	Cys	Pro	Asn	Val	Cys	Asn
259	Gly	Gly	Gly	Phe	Met	Gly	Gly	His	Gln	Gln	Tyr	Asn	Leu	Tyr

compounds (**12a**−**c**) seem to be attractive starting points for further optimization work thus allowing structure-based introduction of substituents that address defined residues in the vicinity of the phenyl binding pocket. We therefore envision that these molecules offer unique opportunities for development of active site-directed inhibitors of not only PTP1B but also other PTPs. In particular, these three scaffolds could be used to address subtle differences in this otherwise conserved region of PTPs. However, for ease of comparison, we have kept the six-membered ring throughout this optimization work. Of note, the inhibitory profile of **12b**[14] against a set of PTPs is almost the same as that of the naphthalene derivative compound **3l**.[4] Thus, although compound **12b** retains the features of a general PTP inhibitor, it shows some selectivity for PTP1B, which also was the case for the naphthyl- and indole-based compounds **3l**−**n**.

We have previously used the fused aminothiophene scaffold represented by compound **12b** for initial validation of two strategies to obtain selectivity for PTP1B by utilizing (i) steric fit/steric hindrance in the area defined by residues 258 and 259[20] and (ii) attraction/repulsion toward residue 48.[14] For a comprehensive list of residues in different PTPs, see Table 4. To further increase our repertoire of synthetic scaffolds for structure-based design of selective PTP inhibitors, we next investigated the potential of substitution of the 6-position, thereby potentially addressing residue 48. Introduction of different heteroatoms in the 6-position of compound **12b** afforded compounds **12d**−**g**, which were all equipotent with **12b** and displayed the same inhibitor profile. Importantly, introduction of a carbonyl in the same 6-position affording **12i** gave a 6-fold increase in potency toward PTP1B and noteworthy also PTPα—which has an asparagine in position 48—when compared to compound **12b**. Reduction of the carbonyl in **12i** to a hydroxy group yielding racemic **12j** only slightly influenced the potency toward PTP1B, although a 4-fold decrease in potency toward PTPα was observed. This tendency was also seen for the more bulky dioxolane **12k**. As a starting point for further optimization toward more potent and selective compounds, the tetrahydrobenzo[*b*]thiophene scaffold represented by compound **12i** offers a unique possibility for introduction of substituents in the 5- and 7-positions. This will allow us to address at least two areas: one represented by residues 24, 254, 258, 259, and 48 and the other by residues 47, 46, 45, 44, and 36.

Fused Amino-Thiophenes with Basic Nitrogen. To further exploit our observation that introduction of a correctly positioned basic nitrogen in compound **12b** resulted in a highly selective inhibitor of PTP1B,[14] we next decided to use **12h** as a starting point for further optimization for potency. Following the reasoning above, it was of particular interest that low energy conformation calculations and FlexX docking indicated that N-alkylated analogues of compound **12h** could address

Table 5. Inhibitory Effect of Analogues of **12h** on PTP1B and TC-PTP



		K_i (μM)		
compd	R_1	PTP1B pH 5.5	PTP1B pH 7	TC-PTP pH 7
12h	H	0.29	4.7	9.4
22a	$-CH_3$	1.0	11	22
22b	$-CH_2$-Ph	1.0	14	24
22c	$-CH_2$-(3-MeO-Ph)	0.7	8.3	ND
22d	$-CH_2$-(2-naphthyl)	ND	42	34
22e	$-CH_2$-(2-pyridyl)	1.0	23	48
22f	$-CH_2$-(3-pyridyl)	0.9	12	ND
22g	$-CH_2$-(4-pyridyl)	1.1	12	ND
22h	$-CH_2$-(2-quinolyl)	1.8	29	ND
22i	$-(CH_2)_2$-Ph	0.27	3.2	7.2
22j	$-(CH_2)_2$-(1-naphthyl)	ND	11	11
22k	$-(CH_2)_2$-(4-biaryl)	ND	8	12
22l	$-(CH_2)_2$-(4-BnO-Ph)	ND	15	10
22m	$-(CH_2)_2$-(2-BnO-Ph)	ND	16	17
22n	$-(CH_2)_2$-(3-thienyl)	ND	9	12
22o	$-(CH_2)_2$-(2-pyridyl)	ND	12	19
22p	$-(CH_2)_2$-(4-pyridyl)	ND	8.7	13
22q	$-(CH_2)_2-CH_2$-Ph	ND	7.3	12
22r	$-(CH_2)_2$-CH-$(Ph)_2$	ND	5.9	5.8

Table 6. Selectivity Profile of Compound **22i**

		K_i (μM)					
compd	R1	PTP1B pH 5.5	PTP1B pH 7	TC-PTP pH 7	PTPα pH 7	LAR pH 7	CD45 pH 7
22i	$-(CH_2)_2$-Ph	0.27	3.2	7.2	2100	2100	1400

the cleft surrounded by Arg24, Arg254, Met258, Gln259, and Asp48 in PTP1B (the 258/259 gateway) but would be prevented from binding to PTPs with bulky side chains in position 259 (PTP1B numbering). Consequently, such inhibitors should have improved affinity for PTP1B and the closely related TC-PTP and almost the same or lower affinity as compound **12h** against PTPs with a blocked 258/259 gateway. To our surprise, introduction of a variety of hydrophobic methylene aryl side chains affording compounds **22b**−**h** (Table 5) resulted in a 3-fold decrease in potency toward PTP1B, although still showing the same good selectivity as seen with **12h** (data not shown) independent of the nature of the aryl group. In the corresponding ethylene aryl series **22i**−**p**, compound **22i** showed the same potency and selectivity for PTP1B (see Table 6) as compound **12h**, whereas all other analogues showed a 3−4-fold decrease in potency. Further extension of the side chain affording propylene mono- and diphenyl compounds **22q**,**r**, respectively, also resulted in loss of potency.

Puzzled by the lack of increased potency that was seen during the optimization of the tetrahydropyridine series based on compound **12h**, we have obtained X-ray crystal data of compounds **22a**,**d**,**i**,**j**,**n** with PTP1B. The cata-

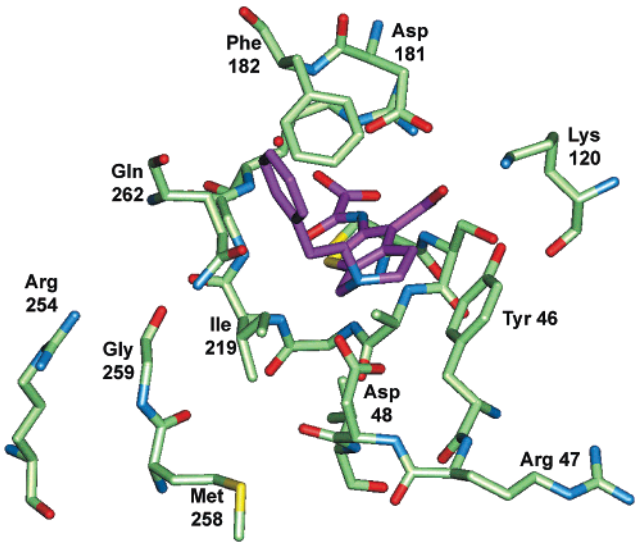


Figure 4. X-ray structure (1.89 Å resolution) of PTP1B cocrystallized with compound **22i**. The binding mode of compound **22i** in the active site pocket of PTP1B is shown. Atoms are colored according to atom type (carbon in light green and violet for compound **22i**, oxygen in red, sulfur in yellow, and nitrogen in blue).

lytic site of the enzyme with the bound inhibitor **22i** at 1.89 Å is shown in Figure 4. All interactions involving the oxalylamide and the *ortho*-carboxylic acid groups with the P loop and Lys120, respectively, were found to be the same as those previously described for compound **12h**.[14] Surprisingly, and in contrast to the Flex X docking and modeling results described above, the nitrogen substituents did not address the 258/259 gateway but instead pointed in the direction of Phe182. Importantly, all compounds showed an unfavorable conformation of the piperidine ring, thus explaining the observed decrease in potency. The same conformation of the piperidine ring was also observed for compound **22i**. However, the resulting loss in potency was apparently compensated for by favorable van der Waals contacts with the aromatic ring of Phe182, resulting in similar overall potency as compound **12h**. Hence, although our initial attempts were unsuccessful regarding improvement of the potency against PTP1B by substituting at the basic nitrogen, it seems conceivable that we have, fortuitously, identified a novel strategy for further optimizations. We hypothesize that compound **22i** could be an attractive stepping stone for development of inhibitors that address the WPD loop. In this context, it is of interest that recent studies have demonstrated that an insulin receptor tyrosine kinase-derived peptide interacts with Phe182 in PTP1B.[26]

Recently, significant progress has been made in several other laboratories toward the development of both peptide and nonpeptide, nonphosphorus inhibitors of PTP1B with K_i values in the nanomolar range.[8,27−29]

Table 7. Pharmacokinetic Parameters of Selected PTP1B Inhibitors

compd	$t_{1/2,iv}$ (min)	V_{ss} (L/kg)	Cl (mL/min/kg)	f_{po} (%)
12d	78		17	85
12h	168	0.9	4.4	56
22i	169	0.9	6.4	13

Although some of the reported compounds had relatively high molecular weights and showed variable selectivity against other PTPs, ranging from a modest 5−6-fold (PTP-LAR and PTP-PEST) to 75- (CD45) and 175-fold (PTPα), they have advanced our understanding of the structural requirements for design of potent and selective PTPs inhibitors. We believe that the structural knowledge obtained in these previous studies can be incorporated in and applied to our novel synthetic scaffold.

Pharmacokinetic and Cellular Uptake. Initial screening of compounds representing both the pyran and the tetrahydropyridine series for both intravenous and oral pharmacokinetics was carried out in rats (Table 7). Noteworthy, compound **12d** exhibited a reasonable acceptable plasma concentrations−time profile with an iv $t_{1/2}$ of 1.3 h and a bioavailability estimated to be 85%, despite a low/intermediate lipophilicity (clog$P$ −0.1) in the range not commonly associated with good prospects for oral bioavailability. The tetrahydro-pyridine analogues **12h** and **22i** showed a similar pharmacokinetic profile although with a lower bioavailability for the more lipophilic compound **22i**. An iv $t_{1/2}$ of around 3 h was found for both compounds **12h** and **22i**. Inspired by the good pharmacokinetics, selected compounds were tested for augmentation of insulin-stimulated 2-deoxyglucose (2-DOG)[30] uptake into C2C12 cells. Disappointingly, none of the tested compounds enhanced accumulation of 2-DOG. This may result from limited ability to cross a cell membrane indicated by low transport across cell monolayers (Caco-2 or MDCK).[31,32] The permeability coefficients were generally less than 1 cm/s (data not shown).

These findings are most likely due to the charged nature of the compounds, which possess two carboxy groups and, in the case of tetrahydropyridine-based inhibitors, also a basic nitrogen. At physiological pH, the carboxy groups and the basic nitrogen create zwitterions, which have very low probability of crossing cell membranes via passive diffusion. Nonetheless, several compounds in these series show acceptable oral bioavailability as described above. To circumvent this problem, we analyzed prodrug esters of compound **3n, 12h**, and **22i**. As an illustrative example of the prodrug concept, compound **11h** (Figure 5) exhibited significantly enhanced penetration of MDCK cell monolayers and was fully cleaved in rat serum to the parent compound **12h** within minutes. At a 100 μM concentration, compounds **2j** and **17i** now showed augmentation

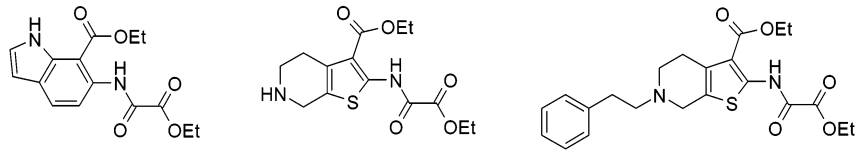


Figure 5. Prodrugs.

of insulin-stimulated 2-DOG[30] uptake into C2C12 cells of almost 100 and 70% of maximum insulin response, respectively.

Conclusions

Using a structure-based lead optimization strategy, we have used the relatively weak, general PTP inhibitor OBA—obtained via high throughput screening—to develop several versatile, novel PTP inhibitor scaffolds represented by compounds **8a**, **12i**,**j**, and **22i**. Of note, the molecular weight of these scaffolds was kept low to allow further optimization for potency, selectivity, and oral activity of inhibitors of different PTPs.

Experimental Section

General Procedures. Melting points were determined on a Buchi 535 capillary melting point apparatus and are uncorrected. Elemental analyses were measured in the Analytical Department of Novo Nordisk A/S and are within 0.4% of theoretical values unless otherwise indicated. MS/SP spectra were recorded on a Therme Finnigan TSQ 70B. ^{1}H NMR spectra were determined on Bruker AM200 or AM300 spectrometers. Chemical shifts are given in δ values (ppm) using trimethylsilane (TMS) as the internal standard. Column chromatography was performed using silicagel (MN Kiselgel 60, Art. 815 380). The biological assays were performed as previously described.[4] 2-DOG uptake was measured by the zero-trans method using [^{3}H]-2-deoxy-D-glucose (2-DOG, 100 μmol/L, 1 μCi, Amersham, U.K.) essentially as described by Berridge and Tan.[30]

Compounds **9a**−**m**, **11b**, **13a**,**b**, **14**, **15a**,**i**, and **16b** were either commercially available or synthesized as described in the literature. 2-Amino-thiophenes **10a**−**c** and 3-amino-thiophenes **6a**−**e** were commercially available. 3-Amino-thiophenes **6g**−**k**,**m**−**o** were synthesized in a similar way as described by Hartmann and Liebscher.[15]

General Preparation Method A. 2-(Oxalylamino)benzoic Acid (3a). To a stirred solution of anthranilic acid (20.1 g, 0.15 mol) in dry THF (250 mL) was added dropwise ethyl oxalyl chloride (10.0 g, 0.073 mol). The mixture was stirred at room temperature for 15 min and filtered, and the solvent was evaporated affording crude 16.4 g (94%) of 2-(ethoxyoxalylamino)benzoic acid (**2a**) as an oil, which was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (t, 3H), 4.31 (q, 2H), 7.23 (t, 1H), 7.65 (t, 1H), 8.03 (d, 1H), 8.56 (d, 1H), 12.6 (s, 1H, N***H***COCOOEt).

To a solution of **2a** (10.0 g, 42 mmol) in ethanol (350 mL) was added a solution of sodium hydroxide (3.7 g, 92 mmol) in water (100 mL). After it was stirred at room temperature for 60 h, concentrated HCl was added to pH 1 and the precipitate was filtered off, washed with water (3 × 100 mL) and diethyl ether (3 × 80 mL), and dried affording 7.1 g (81%) of **3a**; mp 214−215 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.20 (t, 1H), 7.62 (t, 1H), 8.04 (d, 1H), 8.67 (d, 1H), 12.6 (s, 1H, N***H***COCOOH). Anal. ($C_9H_7NO_5$·0.2H_2O) C, H, N.

2-Methyl-6-(oxalylamino)benzoic Acid (3b). Step 1. 2-(Ethoxyoxalylamino)-6-methylbenzoic Acid (2b). Yield 0.2 g (4%) of **2b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (t, 3H), 2.62 (s, 3H), 4.42 (q, 2H), 7.10 (d, 1H), 7.47 (t, 1H), 8.43 (d, 1H), 11.5 (s, 1H, N***H***COCOOEt).

Step 2. 2-Methyl-6-(oxalylamino)benzoic Acid (3b). Yield 70 mg (79%) of **3b**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.39 (s, 3H), 7.04 (d, 1H), 7.36 (t, 1H), 8.08 (d, 1H), 10.94 (bs, 1H, N***H***COCOOH). Anal. ($C_{10}H_9NO_5$·1.4H_2O) C, H, N.

5-Methyl-6-(oxalylamino)benzoic Acid (3c). Step 1. 2-(Ethoxyoxalylamino)-3-methylbenzoic Acid Ethyl Ester (2c). Yield 2.1 g (66%) of **2c**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, 3H), 1.44 (t, 3H), 2.30 (s, 3H), 4.37 (q, 2H), 4.43 (q, 2H), 7.25 (t, 1H), 7.45 (d, 1H), 7.85 (d, 1H), 10.52 (s, 1H, N***H***COCOOEt).

Step 2. 5-Methyl-6-(oxalylamino)benzoic Acid (3c). Yield 20 mg (75%) of **3c**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.20 (s, 3H), 7.2−7.7 (m, 3H), 10.5 (s, 1H, N***H***COCOOH), 12.9 (bs, 1H). Anal. ($C_{10}H_9NO_5$) C, H, N.

2-(Methyloxalylamino)benzoic Acid (3d). Step 1. 2-(Ethoxyoxalylmethylamino)benzoic Acid Methyl Ester (2d). Yield 3.2 g (80%) of **2d**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 (t, 3H), 3.33 (s, 3H), 3.92 (s, 3H), 3.96 (q, 2H), 7.35 (d, 1H), 7.47 (t, 1H), 7.60 (t, 1H), 8.02 (d, 1H).

Step 2. 2-(Methyloxalylamino)benzoic Acid (3d). Yield 0.8 g (30%) of **3d**; mp 144−144.5 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.18 (s, 3H), 7.44 (d, 1H), 7.51 (t, 1H), 7.65 (t, 1H), 7.94 (d, 1H), 13.1 (bs, 1H, N***H***COCOOH). Anal. ($C_{10}H_9NO_5$) C, H, N.

2-(Oxalylamino)thiophene-3-carboxylic Acid (3e). Step 1. 2-(Ethoxyoxalylamino)thiophene-3-carboxylic Acid Ethyl Ester (2e). Yield 3.6 g (45%) of **2e**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (t, 3H), 1.44 (t, 3H), 4.39 (q, 2H), 4.45 (q, 2H), 6.86 (d, 1H), 7.27 (d, 1H), 12.25 (s, 1H, N***H***COCOOEt).

Step 2. 2-(Oxalylamino)thiophene-3-carboxylic Acid (3e). Yield 1.66 g (84%) of **3e**; mp 225−228 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.05 (d, 1H), 7.18 (d, 1H), 12.1 (bs, 1H, N***H***COCOOH), 13.25 (bs, 1H). Anal. ($C_7H_7NO_5S$·1.25 H_2O) C, H, N.

3-(Oxalylamino)thiophene-4-carboxylic Acid (3f). Step 1. 4-(Ethoxyoxalylamino)thiophene-3-carboxylic Acid Methyl Ester (2f). Yield 0.3 g (60%) of **2f**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (t, 3H), 3.93 (s, 3H), 4.43 (q, 2H), 8.07 (d, 1H), 8.12 (d, 1H), 12.5 (s, 1H, N***H***COCOOEt).

Step 2. 3-(Oxalylamino)thiophene-4-carboxylic Acid (3f). Yield 0.1 g (45%) of **3f**; mp 210 °C (decomposition). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.02 (d, 1H), 8.35 (d, 1H), 11.5 (s, 1H, N***H***COCOOH), 13.8 (bs, 1H). Anal. ($C_7H_5NO_5S$·0.25H_2O) C, H, N.

3-(Oxalylamino)thiophene-2-carboxylic Acid, Monosodium Salt (3g). Step 1. 3-(Ethoxyoxalylamino)thiophene-2-carboxylic Acid Methyl Ester (2g). Yield 7.5 g (92%) of **2g**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (t, 3H), 3.94 (s, 3H), 4.44 (q, 2H), 7.51 (d, 1H), 8.13 (d, 1H), 11.6 (s, 1H, N***H***COCOOEt).

Step 2. 3-(Oxalylamino)thiophene-2-carboxylic Acid, Monosodium Salt (3g). Yield 3.0 g (100%) of **3g**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.91 (d, 1H), 8.09 (d, 1H), 11.67 (s, 1H, N***H***COCOOH). Anal. ($C_7H_7NO_5SNa$·1.5H_2O) C, H, N.

4-Methyl-3-(oxalylamino)thiophene-2-carboxylic Acid (3h). Step 1. 3-(Ethoxyoxalylamino)-4-methylthiophene-2-carboxylic Acid Methyl Ester (2h). Yield 3.6 g (84%) of **2h**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (t, 3H), 2.25 (s, 3H), 3.88 (s, 3H), 4.44 (q, 2H), 7.16 (s, 1H), 10.25 (s, 1H, N***H***COCOOEt).

Step 2. 4-Methyl-3-(oxalylamino)thiophene-2-carboxylic Acid (3h). Yield 0.4 g (28%) of **3h**; mp 232−234 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.1 (s, 3H), 7.47 (s, 1H), 10.36 (bs, 1H, N***H***COCOOH). Anal. ($C_8H_7NO_5S$·1.5H_2O) C, H, N.

2-(Oxalylamino)-5-iodobenzoic Acid (3i). Step 1. 2-(Ethoxyoxalylamino)-5-iodobenzoic Acid (2i). Yield 23 mg (64%) of **2i**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.32 (t, 3H), 4.32 (q, 2H), 8.00 (dd, 1H), 8.29 (s, 1H), 8.40 (d, 1H), 12.5 (s, 1H, N***H***COCOOEt).

Step 2. 2-(Oxalylamino)-5-iodobenzoic Acid (3i). Yield 106 mg (16%) of **3i**; mp 201 °C (decomposition). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.98 (m, 1H), 8.26 (d, 1H), 8.40 (d, 1H), 12.4 (s, 1H, N***H***COCOOH). Anal. ($C_9H_6NIO_5$·0.75H_2O) C, H, N.

4-(Oxalylamino)biphenyl-3-carboxylic Acid (3j). To a suspension of 5-bromo-2-aminobenzoic acid methyl ester (3.0 g, 13,04 mmol), tetrakis(triphenylphosphine)palladium(0) (0.5 g, 0.44 mmol), toluene (40 mL), and 2 N aqueous Na_2CO_3 (14.8 mL) were added a solution of phenylboronic acid (2.2 g, 17.73 mmol) in MeOH (10 mL) at room temperature. The resulting reaction mixture was heated at reflux temperature for 4 h, cooled, and diluted with water (50 mL). The insoluble matter was filtered off, and the phases were separated. The aqueous

phase was extracted with ethyl acetate (100 mL), and the combined organic phases were washed with water (2 × 80 mL), diluted aqueous ammoniac (80 mL), and saturated aqueous sodium chloride (80 mL). The organic phase was dried ($MgSO_4$), filtered, and evaporated affording 3.4 g of crude **1j**, which was submitted to flash chromatography using EtOAc/heptane (1:3) affording 2.7 g (91%) of 4-aminobiphenyl-3-carboxylic acid methyl ester (**1j**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.90 (s, 3H), 5.75 (bs, 2H, −N***H***$_2$), 6.72 (d, 1H), 7.27 (d, 2H), 7.52 (m, 3H), 8.11 (d, 1H).

Compound **1j** was converted into **3j** by a similar procedure as described for **3a**.

Step 1. 4-(Ethoxyoxalylamino)biphenyl-3-carboxylic Acid Methyl Ester (2j). Yield 1.4 g (39%) of **2j**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (t, 3H), 4.01 (s, 3H), 4.46 (q, 2H), 7.35 (t, 1H), 7.44 (t, 2H), 7.58 (d, 2H), 7.83 (dd, 1H), 8.31 (d, 1H), 8.80 (d, 1H), 12.6 (s, 1H, N***H***COCOOEt).

Step 2. 4-(Oxalylamino)biphenyl-3-carboxylic Acid (3j). Yield 1 g (88%) of **3j**; mp 223−224 °C. ^{1}H NMR (DMSO-d_6): δ 7.39 (t, 1H), 7.51 (t, 2H), 7.69 (d, 2H), 7.99 (d, 1H), 8.29 (m, 1H), 8.71 (d, 1H), 12.5 (s, 1H, N***H***COCOOH), 14.05 (bs, 1H). Anal. ($C_{15}H_{11}NO_5$·1/2H_2O) C, H, N.

3-(Oxalylamino)isonicotinic Acid (3k). Step 1. 3-(Ethoxyoxalylamino)isonicotinic Acid (2k). Yield 0.4 g (46%) of **2k**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.36 (t, 3H), 4.41 (q, 2H), 8.05 (d, 1H), 8.9 (d, 1H), 9.16 (s, 1H).

Step 2. 3-(Oxalylamino)isonicotinic Acid (3k). Yield 80 mg (22%) of **3k**; mp >250 °C. ^{1}H NMR (DMSO-d_6): δ 7.88 (d, 1H), 8.52 (d, 1H), 9.75 (s, 1H), 12.1 (s, 1H, N***H***COCOOH). Anal. ($C_8H_6N_2O_5$); C, H, N.

3-(Oxalylamino)naphthalene-2-carboxylic Acid (3l). Step 1. 3-(Ethoxyoxalylamino)naphthalene-2-carboxylic Acid (2l). Yield 7.6 g (∼100%) of **2l**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.36 (t, 3H), 4.35 (q, 2H), 7.53 (t, 1H), 7.65 (t, 1H), 7.92 (d, 1H), 8.05 (d, 1H), 8.74 (s, 1H), 9.08 (s, 1H), 12.65 (s, 1H, N***H***COCOOEt).

Step 2. 3-(Oxalylamino)naphthalene-2-carboxylic Acid (3l). Yield 1 g (43%) of **3l**; mp 227−228 °C. ^{1}H NMR (DMSO-d_6): δ 7.48 (t, 1H), 7.60 (t, 1H), 7.87 (d, 1H), 8.02 (d, 1H), 8.70 (s, 1H), 9.11 (s, 1H), 12.7 (s, 1H, N***H***COCOOH). Anal. ($C_{13}H_9NO_5$) C, H, N.

6-(Oxalylamino)-1*H*-indole-7-carboxylic Acid (3m). 6-Amino-1*H*-indole-7-carboxylic acid ethyl ester (**1m**) was prepared as described in ref 33.

Step 1. 6-(Ethoxyoxalylamino)-1*H*-indole-5-carboxylic Acid Ethyl Ester (2m). Yield 8.7 g (∼100%) of **2m**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (t, 3H), 1.51 (t, 3H), 4.46 (q, 2H), 4.51 (q, 2H), 6.58 (bs, 1H), 7.33 (m, 1H), 8.48 (s, 1H), 9.25 (s, 1H), 10.4 (bs, 1H), 13.1 (bs, 1H, N***H***COCOOEt).

Step 2. 6-(Oxalylamino)-1*H*-indole-7-carboxylic Acid (3m). Yield 1.34 g (82%) of **3m**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.51 (m, 1H), 7.33 (m, 1H), 7.84 (d, 1H), 8.39 (d, 1H), 11.03 (s, 1H, N***H***), 12.8 (s, 1H, N***H***COCOOH), 14.1 (bs, 1H). Anal. ($C_{11}H_7N_2O_5Na$·1.5H_2O) C, H, N.

6-(Oxalylamino)-1*H*-indole-5-carboxylic Acid (3n). 6-Amino-1*H*-indole-5-carboxylic acid ethyl ester (**1n**) was prepared as described in ref 33.

Step 1. 6-(Ethoxyoxalylamino)-1*H*-indole-7-carboxylic Acid Ethyl Ester (2n). Yield 2.42 g (81%) of **2n**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (t, 3H), 1.57 (t, 3H), 4.44 (q, 2H), 4.61 (q, 2H), 6.56 (t, 1H), 7.26 (m, 1H), 7.84 (d, 1H), 8.55 (d, 1H), 9.65 (bs, 1H), 12.5 (bs, 1H, N***H***COCOOEt).

Step 2. 6-(Oxalylamino)-1*H*-indole-5-carboxylic Acid (3n). Yield 0.73 g (99%) of **3n**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.55 (s, 1H), 7.44 (m, 1H), 8.39 (s, 1H), 8.80 (s, 1H), 11.5 (s, 1H, N***H***), 12.8 (s, 1H, N***H***COCOOH), 13.25 (bs, 1H). Anal. ($C_{11}H_7N_2O_5Na$·1.5H_2O) C, H, N.

General Preparation Method B. 3-Amino-5-(4-methoxyphenyl)thiophene-2-carboxylic Acid Methyl Ester (6g). To a solution of sodium methoxide (0.7 g, 12.5 mmol) in MeOH (50 mL) was added mercapto-acetic acid methyl ester (1 mL, 11.2 mmol) and **5g** (2.0 g, 8.94 mmol). The reaction mixture was refluxed for 16 h, and the volatiles were evaporated. To the residue was added water (100 mL), and the mixture was extracted with Et_2O (2 × 50 mL). The organic phase was washed with brine (50 mL), dried (Na_2SO_4), filtered, and evaporated, which afforded 2.5 g (95%) of **6g**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.71 (s, 3H, −OC***H***$_3$), 3.79 (s, 3H, −OC***H***$_3$), 6.55 (bs, 2H, −N***H***$_2$), 6.84 (s, 1H), 6.98 (d, 2H), 7.54 (d, 2H).

The following compounds (**6h**−**o**) were prepared in a similar way as described for **6g**.

3-Amino-5-(4-phenoxyphenyl)thiophene-2-carboxylic Acid Methyl Ester (6h). Yield 8.53 g (66%) of **6h**; sp/ms *m*/*z* 325. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.85 (s, 3H, −OC***H***$_3$), 5.45 (bs, 2H, −N***H***$_2$), 6.68 (s, 1H), 6.99 (m, 4H), 7.15 (t, 1H), 7.36 (t, 2H), 7.54 (d, 2H).

3-Amino-5-(4-benzyloxyphenyl)thiophene-2-carboxylic Acid Methyl Ester (6i). Yield 6.8 g (72%) of **6i**; sp/ms *m*/*z* 339. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.85 (s, 3H, −OC***H***$_3$), 5.09 (s, 2H), 5.44 (bs, 2H, −N***H***$_2$), 6.67 (s, 1H), 7.01 (d, 2H), 7.40 (m, 5H), 7.52 (d, 2H).

3-Amino-5-(3-nitrophenyl)thiophene-2-carboxylic Acid Methyl Ester (6k). Yield 1.3 g (20%) of **6k** after silicagel column chromatography using a mixture of EtOAc/heptane (1:2) as eluent. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.74 (s, 3H, −OC***H***$_3$), 6.63 (bs, 2H, −N***H***$_2$), 7.16 (s, 1H), 7.72 (t, 1H), 8.05 (d, 1H), 8.20 (d, 1H), 8.32 (s, 1H).

3-Amino-5-(3-methoxyphenyl)thiophene-2-carboxylic Acid Methyl Ester (6m). Yield 7.1 g (53%) of **6m** after silicagel column chromatography using a mixture of EtOAc/heptane (1:2) as eluent. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 3H, −OC***H***$_3$), 5.46 (bs, 2H, −N***H***$_2$), 6.75 (s, 1H), 6.88 (dd, 1H), 7.08 (m, 1H), 7.16 (d, 1H), 7.28 (t, 1H).

3-Amino-5-(3,4-dimethoxyphenyl)thiophene-2-carboxylic Acid Methyl Ester (6n). Yield 0.8 g (12%) of **6n** after silicagel column chromatography using a mixture of EtOAc/heptane (1:2) as eluent. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 3H, −OC***H***$_3$), 3.90 (s, 3H, −OC***H***$_3$), 3.91 (s, 3H, −OC***H***$_3$), 5.48 (bs, 2H, −N***H***$_2$), 6.65 (s, 1H), 6.84 (d, 1H), 7.04 (d, 1H), 7.14 (d, 1H).

3-Amino-5-(3,5-dimethoxyphenyl)thiophene-2-carboxylic Acid Methyl Ester (6o). Yield 2.5 g (95%) of **6o**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 6H, 2x −OC***H***$_3$), 3.85 (s, 3H, −COOC***H***$_3$), 5.46 (bs, 2H, −N***H***$_2$), 6.45 (m, 1H), 6.71 (m, 1H), 6.73 (s, 1H).

5-(4-Fluorophenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid (8d). To an ice-cooled solution of 5-(4-fluorophenyl)-3-aminothiophene-2-carboxylic acid methyl ester (**6d**) (1.0 g, 4.0 mmol) and triethylamine (11.1, 80 mmol) in dry THF (40 mL) was added dropwise ethyl oxalyl chloride (1.2 g, 9.0 mmol). After it was stirred for 2 h, the reaction mixture was filtered and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 and washed with 0.1 N HCl (2 x-discard). The organic phase was dried ($MgSO_4$) and filtered, and the solvent was evaporated. The residue was submitted to flash chromatography using toluene/ethyl acetate (19:1) as eluent, to give 1.19 g (85%) of 5-(4-fluorophenyl)-3-(ethoxyoxalylamino)thiophene-2-carboxylic acid ethyl ester (**7d**).

To a solution of **7d** (1.19 g, 3.4 mmol) in MeOH (150 mL) was added 2 N NaOH (20 mL). The reaction mixture was stirred at 60 °C for 18 h. The volatiles were evaporated, and to the residue were added water and 1 N HCl to pH 1. The aqueous phase was extracted with a mixture of CH_2Cl_2/2-propanol. The organic phase was dried ($MgSO_4$) and filtered, and the solvent was evaporated. The residue was recrystallized from methanol/water to give 619 mg (67%) of **8d**; mp 221−222 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.30 (t, 2H), 7.76 (m, 2H), 8.26 (s, 2H), 11.6 (s, 1H, N***H***CO). Anal. ($C_{13}H_8FNO_5S$·1/2H_2O) C, H, N.

The following compounds were prepared by the method described for the preparation of compound **8d**.

3-(Oxalylamino)-5-phenylthiophene-2-carboxylic Acid, Monosodium Salt (8a). Yield 2.0 g (91%) of **8a**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.39−7.51 (m, 3H), 7.67 (d, 2H), 8.37 (s, 1H), 11.8 (s, 1H, N***H***COCOOH). Anal. ($C_{13}H_8NO_5SNa$·1.6H_2O) C, H, N.

4-(Oxalylamino)-[2,3]-bithiophenyl-5-carboxylic Acid (8b). Yield 0.8 g (91%) of **8b**; mp 220−222 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.48 (m, 1H), 7.69 (m, 1H), 8.04 (m, 1H), 8.20 (s, 1H), 11.54 (s, 1H, N***H***COCOOH), 13.8 (bs, 1H). Anal. ($C_{11}H_7NO_5S_2$) C, H, N.

5-(4-Isobutylphenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid (8c). Yield 1.5 g (24%) of **8c**; mp 201−202 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87 (d, 6H), 1.85 (m, 1H), 2.47 (d, 2H), 7.23 (d, 2H), 7.55 (d, 2H), 8.32 (s, 1H), 11.82(s, 1H, N***H***COCOOH). Anal. ($C_{17}H_{17}NO_5S\cdot0.33H_2O$) C, H, N.

5-(4-Chlorophenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid, Monosodium Salt (8e). Yield 0.7 g (40%) of **8e**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.52 (d, 2H), 7.69 (d, 2H), 8.36 (s, 1H), 11.8 (s, 1H, N***H***COCOOH). Anal. ($C_{13}H_7ClNO_5SNa\cdot1H_2O$) C, H, N.

5-(4-Hydroxyphenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid, Monosodium Salt (8f). Step 1. 5-(4-Benzyloxyphenyl)-3-(ethoxyoxalylamino)thiophene-2-carboxylic Acid Methyl Ester (7f). To a solution of **6f** (10 g, 29.5 mmol) in dry THF (280 mL) at 0 °C was added tetraethylammonium (TEA, 17 mL) followed by dropwise addition of ethyloxalyl chloride (3.95 mL, 35.5 mmol). The reaction mixture was stirred at room temperature for 16 h and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (150 mL), washed with water (2 × 75 mL), dried ($MgSO_4$), filtered, and evaporated, which afforded 10.0 g (77%) of **7f**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (t, 3H), 3.93 (s, 3H, −OC***H***$_3$), 4.44 (q, 2H), 5.11 (s, 2H), 6.99 (d, 2H), 7.30−7.43 (m, 5H), 7.55 (d, 2H), 8.23 (s, 1H), 11.6 (s, 1H, N***H***COCOOEt).

Step 2. 3-(Ethoxyoxalylamino)-5-(4-hydroxyphenyl)-thiophene-2-carboxylic Acid Methyl Ester (7ff). To a solution of **7f** (9.26 g, 21.1 mmol) in 33% HBr in AcOH (500 mL) was added *n*-hexyl-tri-*n*-butylphosphoniumbromide (0.99 g), and the resulting mixture was stirred at room temperature for 4.5 h. The volatiles were evaporated, and the residue was crystallized from hot CH_2Cl_2/MeOH by addition of petroleum ether to initiate crystallization. The precipitate was filtered off and dried yielding 5.4 g (73%) of **7ff**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.32 (t, 3H), 3.85 (s, 3H, −OC***H***$_3$), 4.32 (q, 2H), 6.84 (d, 2H), 7.53 (d, 2H), 8.09 (s, 1H), 10.03 (bs, 1H), 11.35 (s, 1H, N***H***COCOOEt).

Step 3. 5-(4-Hydroxyphenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid, Monosodium Salt (8f). To a solution of **7ff** (270 mg, 0.77 mmol) in EtOH (50 mL) was added 1 N NaOH (20 mL). The reaction mixture was stirred at room temperature for 16 h. The volatiles were evaporated, and to the residue was added water (30 mL) followed by washing with CH_2Cl_2 (25 mL). The aqueous phase was acidified with 1 N HCl to pH 1, and the precipitate was filtered off, washed with water (3 × 25 mL), and dried, which yielded 204 mg (86%) of **8f**; mp 205−206 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.85 (d, 2H), 7.53 (d, 2H), 8.15 (s, 1H), 10.05 (bs, 1H), 11.6 (s, 1H, N***H***COCOOH). Anal. ($C_{13}H_9NO_6SNa\cdot0.75H_2O$) C, H, N.

5-(4-Methoxyphenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid (8g). Yield 0.7 g (88%) of **8g**; mp 220−221 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.81 (s, 3H), 7.02 (d, 2H), 7.65 (d, 2H), 8.18 (s, 1H), 11.5 (s, 1H, N***H***COCOOH), 13.75 (bs, 1H). Anal. ($C_{14}H_{11}NO_6S\cdot0.4H_2O$) C, H, N.

3-(Oxalylamino)-5-(4-phenoxyphenyl)thiophene-2-carboxylic Acid (8h). Yield 0.85 g (37%) of **8h**; mp 230 °C (decomposition). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.09 (t, 4H), 7.18 (t, 1H), 7.43 (t, 2H), 7.74 (d, 2H), 8.26 (s, 1H), 11.5 (s, 1H, N***H***COCOOH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 100.1, 117.4, 118.3, 119.6, 120.2, 124.5, 125.0, 126.3, 127.4, 128.2, 130.2, 130.9, 142.0, 143.1, 148.4, 156.6, 158.9, 162.0, 165.3. Anal. ($C_{19}H_{13}NO_6S\cdot1.25H_2O$) C, H, N.

5-(4-Benzyloxyphenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid (8i). Yield 0.88 g (67%) of **8i**; mp 210 °C (decomposition). ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.17 (s, 2H), 7.12 (d, 2H), 7.31−7.45 (m, 5H), 7.66 (d, 2H), 8.20 (s, 1H), 11.5 (s, 1H, N***H***COCOOH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 68.2, 109.9, 114.5, 114.7, 123.9, 124.2, 126.1, 126.5, 126.7, 127.3, 135.5, 141.0, 147.2, 153.6, 158.2, 159.8, 163.3. Anal. ($C_{20}H_{15}NO_6S$) C, H, N.

5-(4-Carboxymethoxyphenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid, Trisodium Salt (8j). To a solution of **7ff** (307 mg, 1.0 mmol) and K_2CO_3 (166 mg, 1.2 mmol) in DMF (5 mL) was added 2-bromoacetamide (165 mg, 1.2 mmol). After it was stirred at 50 °C for 16 h, the reaction mixture was quenched by addition of water, and the precipitate 5-(4-carbamoylmethoxy-phenyl)-3-(ethoxyoxalylamino)thiophene-2-carboxylic acid methyl ester (70 mg) was isolated by filtration.

The aqueous phase was acidified with 1 N HCl to pH 1−2 and the semihydrolyzed product, 5-(4-carbamoylmethoxyphenyl)-3-(oxalylamino)thiophene-2-carboxylic acid methyl ester (**7fff**) (300 mg), was isolated by filtration. To a suspension of **7fff** (295 mg, 0.78 mmol) in MeOH (5 mL) and water (5 mL) was added 1 N NaOH (2 mL). After it was stirred for 5 days, the precipitate was filtered off and dried affording 105 mg (88%) of **8j**; mp >300 °C. ^{1}H NMR (300 MHz, D_2O): δ 4.20 (s, 2H), 6.68 (d, 2H), 7.38 (d, 2H), 7.80 (s, 1H). MS (ESI) *m*/*z* 432 (M + 1, 3Na), 410 (M + 1, 2Na), 366 (M + 1). Anal. ($C_{15}H_{12}NO_{10}SNa_3$) C, H, N.

5-(3-Nitrophenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid (8k). Step 1. 3-(Ethoxyoxalylamino)-5-(3-nitrophenyl)thiophene-2-carboxylic Acid Methyl Ester (7k). To a solution of **6k** (2.5 g, 8.98 mmol) in dry THF (100 mL) at 0 °C was added TEA (3 mL) followed by dropwise addition of a solution of ethyloxalyl chloride (1.4 g, 35.5 mmol) in dry THF (20 mL). The reaction mixture was stirred at room temperature for 16 h and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (150 mL), washed with water (2 × 75 mL), dried ($MgSO_4$), filtered, and evaporated, which afforded 2.5 g (74%) of **7k**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (t, 3H), 3.97 (s, 3H, −OC***H***$_3$), 4.46 (q, 2H), 7.61 (t, 1H), 7.96 (d, 1H), 8.22 (m, 1H), 8.47 (m, 1H), 11.63 (s, 1H, N***H***COCOOEt).

Step 2. 5-(3-Nitrophenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid (8k). To a solution of **7k** (600 mg, 1.56 mmol) in a mixture of water (75 mL) and EtOH (25 mL) was added 1 N NaOH (4.8 mL). The reaction mixture was stirred at room temperature for 48 h. The volatiles were evaporated, and to the residue was added water (30 mL) followed by washing with CH_2Cl_2 (25 mL). The aqueous phase was acidified with 1 N HCl to pH 1, and the precipitate was filtered off, washed with water (3 × 25 mL), and dried, which yielded 0.5 g (94%) of **8k**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.74 (t, 1H), 8.11 (d, 1H), 8.22 (d, 1H), 8.36 (s, 1H), 8.50 (s, 1H), 11.9 (s, 1H, N***H***COCOOH). Anal. ($C_{13}H_7NO_7SNa\cdot1.25H_2O$) C, H, N.

5-(3-Aminophenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid (8l). Step 1. 5-(3-Aminophenyl)-3-(ethoxyoxalylamino)thiophene-2-carboxylic Acid Methyl Ester (7l). Compound **7k** (1.6 g, 4.23 mmol) was dissolved in a mixture of EtOH/THF (200 mL, 1:1), and 10% Pd/C (0.4 g, containing 50% water) was added. The mixture was hydrogenated at atmospheric pressure affording after filtration and evaporation 1.3 g (88%) of **7l**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (t, 3H), 3.93 (s, 3H, −OC***H***$_3$), 4.44 (q, 2H), 6.69 (d, 1H), 6.95 (s, 1H), 7.03 (d, 1H), 7.18 (t, 1H), 8.29 (s, 1H) 11.65 (s, 1H, N***H***COCOOEt).

Step 2. 5-(3-Aminophenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid (8l). To a solution of **7l** (500 mg, 1.44 mmol) in a mixture of water (75 mL) and EtOH (25 mL) was added 1 N NaOH (4.3 mL). The reaction mixture was stirred at room temperature for 48 h. The volatiles were evaporated, and to the residue was added water (30 mL) followed by washing with CH_2Cl_2 (25 mL). The aqueous phase was acidified with 1 N HCl to pH 1, and the precipitate filtered off, washed with water (3 × 25 mL), and dried, which yielded 0.25 g (57%) of **8l**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.61 (dd, 1H), 6.82 (d, 1H), 6.91 (s, 1H), 7.09 (t, 1H), 8.20 (s, 1H), 11.5 (s, 1H, N***H***COCOOH). Anal. ($C_{13}H_{10}N_2O_5S\cdot1/2H_2O$) C, H, N.

5-(3-Methoxyphenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid (8m). Yield 0.4 g (65%) of **8m**; mp 217−218 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.82 (s, 3H), 7.00 (m, 1H),

7.17 (s, 1H), 7.25 (d, 1H), 7.38 (t, 1H), 8.28 (s, 1H), 11.53 (s, 1H, N***H***COCOOH). Anal. ($C_{14}H_{11}NO_6S \cdot 0.75H_2O$) C, H, N.

5-(3,4-Dimethoxyphenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid (8n). Yield 0.8 g (75%) of **8n**; mp 230−231 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.80 (s, 3H), 3.85 (s, 3H), 7.03 (d, 1H), 7.18 (s, 1H), 7.25 (m, 1H), 8.20 (s, 1H), 11.52 (s, 1H, N***H***COCOOH), 13.8 (bs, 1H). Anal. ($C_{15}H_{13}NO_7S \cdot 1H_2O$) C, H, N.

5-(3,5-Dimethoxyphenyl)-3-(oxalylamino)thiophene-2-carboxylic Acid (8o). Yield 1.2 g (96%) of **8o**; mp 223−225 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.81 (s, 6H), 6.56 (t, 1H), 6.77 (m, 2H), 8.29 (s, 1H), 11.6 (s, 1H, N***H***COCOOH). Anal. ($C_{15}H_{13}NO_7S \cdot 1.25H_2O$) C, H, N.

General Preparation Method C. Compounds **10a**−**k** were synthesized in the following way as exemplified with compound **10b**.

2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic Acid *tert*-Butyl Ester (10b). To a solution of **9b** (5.0 g, 0.051 mol), *tert*-butyl cyanoacetate (7.9 g, 0.056 mol), and sulfur (1.8 g, 0.056 mol) in absolute EtOH (150 mL) was added morpholine (6.7 mL, 0.076 mol). The mixture was heated to 50 °C for 16 h, allowed to cool to room temperature, and filtered, and the filtrate was evaporated. The resultant oil was dissolved in ethyl acetate (100 mL), washed with water (2 × 50 mL) and brine (2 × 50 mL), and dried ($MgSO_4$). The solvent was evaporated, and the residue was subjected to flash column chromatography using ethyl acetate/hexanes (1:10) as eluent affording 10.5 g (81%) of **10b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.54 (s, 9H), 1.75 (m, 4H), 2.48 (m, 2H), 2.67 (m, 2H), 5.84 (bs, 2H, −N***H***$_2$).

2-Amino-4,7-dihydro-5*H*-thieno[2,3-c]pyran-3-carboxylic Acid Ethyl Ester (10d). Yield 17.8 g (78%) of **10d**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (t, 3H), 2.82 (m, 2H), 3.91 (t, 2H), 4.27 (q, 2H), 4.55 (m, 2H), 6.04 (bs, 2H, −N***H***$_2$).

2-Amino-4,7-dihydro-5*H*-thieno[2,3-c]thiopyran-3-carboxylic Acid Ethyl Ester (10e). Yield 16.89 g (71%) of **10e**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (t, 3H), 2.86 (t, 2H), 3.02 (t, 2H), 3.56 (s, 2H), 4.26 (q, 2H), 6.01 (bs, 2H, −N***H***$_2$).

2-Amino-6-oxo-4,7-dihydro-5*H*-thieno[2,3-c]thiopyran-3-carboxylic Acid Ethyl Ester (10f). Yield 5.87 g (75%) of **10f**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (t, 3H), 3.00 (m, 1H), 3.14−3.37 (m, 3H), 3.82 (q, 2H, J = 54 Hz, J = 18 Hz), 4.27 (q, 2H), 6.23 (bs, 2H, −N***H***$_2$).

2-Amino-6,6-dioxo-4,5,6,7-tetrahydro-6-thieno[2,3-c]-thiopyran-3-carboxylic Acid Ethyl Ester (10 g). Yield 4.1 g (44%) of **10g**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.21 (t, 3H), 3.17 (t, 2H), 3.30 (t, 2H), 4.16 (q, 2H), 4.20 (s, 2H), 7.40 (s, 2H, −N***H***$_2$).

2-Amino-6-(2′-spiro[1′,3′]dioxolane)-6,7-dihydro-4H-benzo[*b*]thiophen-3-carboxylic Acid Ethyl Ester (10k). Yield 38.5 g (100%) of **10g**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (t, 3H), 1.89 (t, 2H), 2.72 (s, 2H), 2.92 (t, 2H), 4.01 (s, 4H), 4.25 (q, 2H), 6.01 (bs, 2H, −N***H***$_2$).

2-(Oxalylamino)-5,6-dihydro-4*H*-cyclopenta[b]thiophene-3-carboxylic Acid (12a). Step 1. 2-(Ethoxyoxalylamino)-5,6-dihydro-4*H*-cyclopenta[b]thiophene-3-carboxylic Acid Ethyl Ester (11a). To an ice-cooled solution of **10a** (1.0 g, 4.73 mmol) in THF (25 mL) was added TEA (1.0 mL) followed by dropwise addition of ethyl oxalyl chloride (0.71 g, 5.21 mmol). The reaction mixture was stirred at room temperature for 1 h and quenched by addition of water (100 mL). The precipitate was filtered off, washed with water, and dried affording 1.0 g (68%) of **11a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, 3H), 1.44 (t, 3H), 2.40 (m, 2H), 2.89 (m, 4H), 4.37 (q, 2H), 4.44 (q, 2H), 12.25 (bs, 1H, N***H***CO).

Step 2. 2-(Oxalylamino)-5,6-dihydro-4*H*-cyclopenta[b]-thiophene-3-carboxylic Acid (12a). To a suspension of **11a** (0.9 g, 2.89 mmol) in EtOH (5 mL) and water (20 mL) was added NaOH (0.35 g, 8.67 mmol). After it was stirred for 64 h, the volatiles were evaporated and to the aqueous solution was added 1 N HCl to pH 1. The precipitate was filtered off, washed with water (2 × 25 mL), and dried affording 0.5 g (68%) of **12a**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.31 (m, 2H), 2.82 (m, 4H), 12.1 (s, 1H, N***H***COCOOH), 13.1 (bs, 1H). Anal. ($C_{10}H_9NO_5S \cdot 1.2H_2O$) C, H, N.

The following compounds were prepared by the method described for the preparation of compound **11a**.

2-(Ethoxyoxalylamino)-5,6,7,8-tetrahydro-4*H*-cyclohepta[b]thiophene-3-carboxylic Acid Ethyl Ester (11c). Yield 1.4 g (40%) of **11c**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (t, 6H), 1.65 (m, 4H), 1.85 (m, 2H), 2.57 (m, 2H), 3.07 (m, 2H), 4.41 (m, 4H), 12.25 (bs, 1H, N***H***CO).

2-(Ethoxyoxalylamino)-4,7-dihydro-5*H*-thieno[2,3-c]-pyran-3-carboxylic Acid Ethyl Ester (11d). Yield 5.3 g (72%) of **11d**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (t, 3H), 1.45 (t, 3H), 2.92 (t, 2H), 3.95 (t, 2H), 4.39 (q, 2H), 4.45 (q, 2H), 4.72 (s, 2H), 12.4 (bs, 1H, N***H***CO).

2-(Ethoxyoxalylamino)-4,7-dihydro-5*H*-thieno[2,3-c]-thiopyran-3-carboxylic Acid Ethyl Ester (11e). Yield 16.21 g (77%) of **11e**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, 3H), 1.41 (t, 3H), 2.91 (t, 2H), 3.11 (t, 2H), 3.72 (s, 2H), 4.38 (q, 2H), 4.43 (q, 2H), 12.45 (bs, 1H, N***H***CO).

2-(Ethoxyoxalylamino)-6-oxo-4,7-dihydro-5*H*-thieno-[2,3-c]thiopyran-3-carboxylic Acid Ethyl Ester (11f). Yield 1.64 g (31%) of **11f**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (m, 6H), 2.94 (m, 1H), 3.26−3.49 (m, 3H), 3.97 (m, 2H), 4.44 (m, 4H), 12.43 (bs, 1H, N***H***CO).

2-(Ethoxyoxalylamino)-6,6-dioxo-4,7-tetrahydro-5*H*-thieno[2,3-c]thiopyran-3-carboxylic Acid Ethyl Ester (11g). Yield 1.6 g (47%) of **11g**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (m, 6H), 3.26 (t, 2H), 3.60 (t, 2H), 4.26 (s, 2H), 4.44 (q, 4H), 12.4 (bs, 2H, N***H***CO).

2-(Ethoxyoxalylamino)-6-(2′-spiro[1′,3′]dioxolane)-6,7-dihydro-4H-benzo[b]thiophen-3-carboxylic Acid Ethyl Ester (11k). 1,4-Cyclohexanedione monoethylene ketale (**9i**) (Aldrich) was used as starting material affording compound **11k** in a similar way as described for **11a**. Yield 18.5 g (38%) of **11k**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (t, 3H), 1.43 (t, 3H), 1.95 (t, 2H), 2.91 (s, 2H), 3.03 (t, 2H), 4.04 (s, 4H), 4.38 (q, 2H), 4.44 (q, 2H), 12.42 (bs, 1H, N***H***CO).

The following compounds were prepared by the method described for the preparation of compound **12a**.

2-(Oxalylamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic Acid (12b). Yield 190 mg (47%) of **12b**; mp 230−231 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.73 (bs, 4H), 2.63 (bs, 2H), 2.74 (bs, 2H), 12.3 (bs, 1H, N***H***COCOOH), 13.35 (bs, 1H). Anal. ($C_{11}H_{11}NO_5S$) C, H, N.

2-(Oxalylamino)-5,6,7,8-tetrahydro-4*H*-cyclohepta[b]-thiophene-3-carboxylic Acid (12c). Yield 150 mg (22%) of **12c**; mp 209−212 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.56 (m, 4H), 1.80 (m, 2H), 2.71 (m, 2H), 3.05 (n, 2H), 12.2 (bs, 1H, N***H***COCOOH), 13.35 (bs, 1H). Anal. ($C_{11}H_{13}NO_5S$) C, H, N.

2-(Oxalylamino)-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-3-carboxylic Acid (12d). Yield 1.2 g (66%) of **12d**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.50 (s, 2H), 3.84 (t, 2H), 4.64 (s, 2H), 12.3 (bs, 1H, N***H***COCOOH), 13.3 (bs, 1H). Anal. ($C_{10}H_8NO_6S \cdot 1/2H_2O$) C, H, N.

2-(Oxalylamino)-4,7-dihydro-5*H*-thieno[2,3-c]thiopyran-3-carboxylic Acid (12e). Yield 2.09 g (83%) of **12e**; mp 227−228 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.89 (m, 2H), 2.99 (m, 2H), 3.77 (s, 2H), 12.35 (bs, 1H, N***H***COCOOH), 13.5 (bs, 1H). Anal. ($C_{10}H_9NO_5S_2$) C, H, N.

2-(Oxalylamino)-6-oxo-4,7-dihydro-5*H*-thieno[2,3-c]thiopyran-3-carboxylic Acid (12f). 1-Oxo-2,3,5,6-tetrahydro-*4H*-thiopyran-4-one was prepared as described in ref 34. Yield 475 mg (51%) of **12f**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.97 (m, 1H), 3.20−3.28 (m, 3H), 4.07 (q, 2H), 12.4 (s, 1H, N***H***COCOOH). Anal. ($C_{10}H_9NO_6S_2 \cdot 0.2NaCl$) C, H, N.

2-(Oxalylamino)-6,6-dioxo-4,7-dihydro-5*H*-thieno[2,3-c]thiopyran-3-carboxylic Acid (12g). 1,1-Dioxide-2,3,5,6-tetrahydro-*4H*-thiopyran-4-one was prepared as described in ref 35. Yield 470 mg (61%) of **12g**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.37 (dd, 4H), 4.46 (s, 2H), 12.4 (bs, 1H, N*H*COCOOH). Anal. ($C_{10}H_8NO_7S_2Na \cdot 1H_2O$) C, H, N.

2-(Oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (12h). Step 1. 2-Amino-4,5,6,7-

tetrahydrothieno[2,3-c]pyridine-3,6-dicarboxylic Acid Di-*tert*-butyl Ester (19b). To a solution of **13d** (50 g, 0.25 mol), *tert*-butyl cyanoacetate (55 mL, 0.38 mol), and sulfur (8.8 g, 0.286 mol) in absolute ethanol (300 mL) was added morpholine (43.7 mL, 0.50 mol). The mixture was heated to 50 °C for 1 h and cooled, and the precipitate was filtered off and washed with ether (3 × 80 mL). This afforded after drying 69.5 g (78%) of **19b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 9H), 1.52 (s, 9H), 2.77 (bt, 2H), 3.60 (t, 2H), 4.34 (s, 2H), 5.98 (bs, 2H, $-NH_2$).

Step 2. 2-(*tert*-Butoxyoxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3,6-dicarboxylic Acid Di-*tert*-butyl Ester (20b). To a solution of **19b** (40 g, 0.113 mol) in CH_2Cl_2 (350 mL) and TEA (31.5 mL, 0.23 mol) was added imidazol-1-yloxoacetic acid *tert*-butyl ester (44.3 g, 0.23 mol). The reaction mixture was stirred at room temperature for 16 h and evaporated. The residue was dissolved in EtOAc (300 mL) and reevaporated affording a crystallizing oil, which was washed with ether, filtered, and dried affording 37 g (68%) of **20b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.61 (s, 9H), 1.62 (s, 9H), 2.86 (bm, 2H), 3.65 (t, 2H), 4.51 (s, 2H), 12.5 (s, 1H, NHCOCO*t*-Bu).

Step 3. 2-(Oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (12h). Compound **20b** (20 g, 0.041 mol) was dissolved in a mixture of TFA and CH_2Cl_2 (1:3) (188 mL). The reaction was stirred at room temperature for 16 h, and the precipitate was filtered off and washed with ether. After it was dried, this afforded 13.97 g (88%) of **12h**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.03 (bs, 2H), 3.38 (bs, 2H), 4.30 (bs, 2H), 9.4 (bs, 2H, NH_2^+), 12.3 (s, 1H, NHCOCOOH). Anal. ($C_{10}H_{10}N_2O_5S\cdot 1/2TFA$, 1 H_2O) C, H, N.

2-(Oxalylamino)-6-oxo-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic Acid (12i). Step 1. 2-(Ethoxyoxalylamino)-6-oxo-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic Acid Ethyl Ester (11i). Compounf **11k** (17.5 g, 0.046 mol) was dissolved in a mixture of TFA and CH_2Cl_2 (250 mL, 1:3) and stirred for 16 h at room temperature. The solvent was evaporated, and the residue was crystallized from ether (50 mL), filtered, and dried affording 12.1 g (78%) of **11i**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (m, 6H), 2.68 (t, 2H), 3.28 (t, 2H), 3.56 (s, 2H), 4.44 (m, 4H), 12.45 (s, 1H, NHCOCOOEt).

Step 2. 2-(Oxalylamino)-6-oxo-4,5,6,7-tetrahydrobenzo-[b]thiophene-3-carboxylic Acid (12i). To a mixture of **11i** (3.0 g, 0.013 mol) in water (40 mL), ethanol (20 mL), and THF (20 mL) at room temperature was added 1 N NaOH (20.24 mL, 20.24 mmol). After it was stirred at room temperature for 72 h, concentrated HCl was added to pH 3. The precipitate was filtered off, washed with water (2 × 15 mL) and diethyl ether (2 × 15 mL), and dried affording 1.96 g (73%) of **12i**; mp >230 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.58 (t, 2H), 3.17 (t, 2H), 3.56 (s, 2H), 12.3 (s, 1H, NHCOCOOH), 13.5 (bs, 1H). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 24.9, 38.3, 38.6, 113.5, 124.4, 131.8, 145.9, 154.9, 160.5, 166.5. Anal. ($C_{11}H_9NO_6S$) C, H, N.

6-Hydroxy-2-(oxalylamino)-4,5,6,7-tetrahydrobenzo[b]-thiophen-3-carboxylic Acid (12j). Step 1. 2-(Ethoxyoxalylamino)-6-hydroxy-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic Acid Ethyl Ester (11j). To a solution of **11i** (3.50 g, 10.31 mmol) in a mixture of EtOH (40 mL) and CH_2Cl_2 (40 mL) was added $NaBH_4$ (0.12 g, 3.09 mmol), and the resulting mixture was stirred for 16 h, filtered, and evaporated. The residue was subjected to flash column chromatography on silica gel (EtOAc/heptane, 1:1) affording 3.7 g (90%) of **11j**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (q, 6H), 1.76 (bs, 1H), 1.89 (m, 1H), 2.02 (m, 1H), 2.71 (dd, 1H), 2.88 (m, 1H), 3.05 (m, 2H), 4.24 (m, 1H), 4.41 (q, 2H), 4.47 (q, 2H), 12.4 (s, 1H, NHCOCOOEt).

Step 2. 6-Hydroxy-2-(oxalylamino)-4,5,6,7-tetrahydrobenzo[b]thiophen-3-carboxylic Acid (12j). To a suspension of **11j** (0.3 g, 0.88 mmol) in water (10 mL) was added 1 N NaOH (3.1 mL, 3.08 mmol). After it was stirred for 16 h, concentrated HCl was added to pH 1 and the solution was evaporated to 1/2 volume. The precipitate was filtered off, washed with ether (2 × 15 mL), and dried, affording 130 mg (52%) of **12j**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.62 (m, 1H), 1.86 (m, 1H), 2.46 (m, 1H), 2.70 (m, 1H), 2.85 (m, 2H), 3.92 (m, 1H), 4.87 (bs, 1H), 12.3 (bs, 1H, NHCOCOOH), 13.3 (bs, 1H). Anal. ($C_{11}H_{11}NO_6SNa\cdot 2.25H_2O$) C, H, N.

2-(Oxalylamino)-6-(2′-spiro[1′,3′]dioxolane)-6,7-dihydro-4H-benzo[b]thiophen-3-carboxylic Acid (12k). 1,4-Cyclohexanedione monoethylene ketale (Aldrich) was used as starting material. Yield 3.75 g (88%) of **12k**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.82 (t, 2H), 2.83 (s, 2H), 2.88 (t, 2H), 3.93 (s, 4H), 12.3 (s, 1H, NHCOCOOH), 13.4 (bs, 1H). Anal. ($C_{13}H_{13}NO_7S$) C, H, N.

General Preparation Method D. 6-Methyl-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22a). Step 1. 2-Amino-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid Ethyl Ester (16a). 1-Methylpiperidin-4-one (**15a**) (20 g, 0.177 mol), ethyl cyanoacetate (22 g, 0.195 mol), sulfur (6.2 g, 0.195 mol), and morpholine (25 mL) were dissolved in absolute ethanol (250 mL) and heated to 50 °C for 18 h. The reaction mixture was cooled and filtered, and the filtrate was evaporated. The resultant oil was dissolved in ethyl acetate (250 mL), and the precipitate formed was filtered off and washed with water (2 × 80 mL) and ether (2 × 50 mL). This afforded after drying 21.89 g (52%) of **16a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32 (t, 3H), 2.43 (s, 3H), 2.66 (t, 2H), 2.83 (t, 2H), 3.37 (m, 2H), 4.25 (q, 2H), 5.95 (bs, 2H, $-NH_2$).

Step 2. 2-(Ethoxyoxalylamino)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid Ethyl Ester (17a). To a stirred solution of **16a** (1.5 g, 6.24 mmol) and TEA (1.3 mL, 9.36 mmol) in dry THF (50 mL) at 0 °C was added dropwise ethyl oxalyl chloride (0.94 g, 6.87 mmol). The resulting mixture was stirred at room temperature for 2 h and evaporated, and to the residue was added ice water (100 mL). The aqueous phase was extracted with EtOAc (2 × 75 mL), washed with brine (100 mL), dried ($MgSO_4$), and evaporated affording 2.1 g (~100%) of **17a** as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (t, 3H), 2.49 (s, 3H), 2.71 (t, 2H), 2.95 (t, 2H), 3.55 (s, 2H), 4.39 (q, 2H), 12.4 (s, 1H, NHCOCOOEt).

Step 3. 6-Methyl-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22a). To a solution of **17a** (5.0 g, 14.69 mmol) in a mixture of THF/EtOH (80 mL, 1:1) was added 1 N NaOH (44 mL, 44 mmol). After they were stirred at room temperature for 18 h, the volatiles were evaporated and the residue was dissolved in water (100 mL) and washed with EtOAc (2 × 50 mL). To the aqueous phase was added concentrated HCl to pH 3, and the precipitate was filtered off, washed with water (2 × 50 mL) and diethyl ether (2 × 30 mL), and dried affording 3.9 g (94%) of **22a**; mp >250 °C. ^{1}H NMR (300 MHz, MeOH-d_4): δ 2.43 (s, 3H), 2.73 (t, 2H), 3.07 (bt, 2H), 3.53 (s, 2H). Anal. ($C_{11}H_{12}N_2O_5S\cdot 1H_2O$). C, H, N.

The following compounds were prepared by the method described for the preparation of compound **22a**.

6-Benzyl-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22b). Step 1. 6-Benzyl-2-(ethoxyoxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid Ethyl Ester (17b). Yield 17.5 g (48%) of **17b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, 3H), 1.43 (t, 3H), 2.80 (t, 2H), 2.92 (t, 2H), 3.60 (s, 2H), 3.72 (s, 2H), 4.37 (q, 2H), 4.44 (q, 2H), 7.24−7.35 (m, 5H), 12.42 (bs, 1H, NHCOCOOEt).

Step 2. 6-Benzyl-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22b). Yield 2.6 g (29%) of **22b**; mp decomposition. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.98 (bs, 2H), 3.24 (bs, 2H), 4.04 (bs, 2H), 4.23 (bs, 2H), 7.40 (m, 3H), 7.48 (m, 2H), 12.7 (bs, 1H, NHCOCOOH). Anal. ($C_{17}H_{16}N_2O_5S\cdot 1.75H_2O$) C, H, N.

2-(Oxalylamino)-6-phenethyl-4,5,6,7-tetrahydrothieno-[2,3-c]pyridine-3-carboxylic Acid (22i). Step 1. 2-Amino-6-phenethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid Ethyl Ester (16i). Yield 43 g (80%) of **16i**.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (t, 3H), 2.74−2.92 (m, 8H), 3.53 (s, 2H), 4.28 (q, 2H), 5.96 (s, 2H, −N***H***$_2$), 7.18−7.32 (m, 5H).

Step 2. 2-(Ethoxyoxalylamino)-6-phenethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid Ethyl Ester (17i). Yield 4.19 g (∼100%) of **17i**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (t, 3H), 1.43 (t, 3H), 2.78−2.98 (m, 8H), 3.70 (s, 2H), 4.39 (q, 2H), 4.44 (q, 2H), 7.18−7.32 (m, 5H), 12.4 (bs, 1H, −N***H***COCOOEt).

Step 3. 2-(Oxalylamino)-6-phenethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22i). Yield 1.55 g (60%) of **22i**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.08 (m, 4H), 3.38 (m, 2H), 3.49 (bs, 2H), 4.39 (bs, 2H), 7.21−7.33 (m, 5H), 12.25 (bs, 1H, N***H***COCOOH). Anal. ($C_{18}H_{18}N_2O_5S\cdot 1H_2O$) C, H, N.

6-Naphthalen-2-ylmethyl-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22d). Step 1. 1-Naphthalen-2-ylmethylpiperidin-4-one (15d). A solution of 2-bromomethylnaphthalene (0.9 g, 4.07 mmol), piperidine-4-one ethylene ketale (**14**) (0.52 g, 3.88 mmol), K_2CO_3 (1.3 g, 9.48 mmol), and KI (20 mg) in MIBK (50 mL) was heated at reflux for 5 h, cooled, and filtered. The residue was submitted to flash chromatography on silica gel (toluene/EtOAc, 19:1) to afford 0.6 g (58%) of 1-naphthalen-2-ylmethylpiperidin-4-one ethylene ketale, which was hydrolyzed directly in a mixture of concentrated HCl (8 mL) and AcOH (42 mL) at reflux for 18 h. The mixture was poured onto ice water (100 mL), neutralized with 32% NaOH to pH 8, extracted with EtOAc (2 × 50 mL), washed with brine (50 mL), and dried ($MgSO_4$). Evaporation afforded 0.5 g (99%) of **15d**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.45 (t, 4H), 2.77 (t, 4H), 3.75 (s, 2H), 7.44 (m, 2H), 7.51 (dd, 1H), 7.73 (s, 1H), 7.81 (m, 3H).

Step 2. 2-Amino-6-naphthalen-2-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (16d). To a solution of **15d** (0.5 g, 2.09 mmol), *tert*-butyl cyanoacetate (0.32 g, 2.30 mmol), and sulfur (74 mg, 2.30 mmol) in absolute ethanol (50 mL) was added morpholine (0.27 mL, 3.13 mmol). The mixture was heated to 50 °C for 16 h, cooled, and filtered, and the filtrate was evaporated. The resultant oil was dissolved in EtOAc (50 mL), washed with water (2 × 50 mL) and brine (2 × 50 mL), and dried ($MgSO_4$). The solvent was evaporated, and the residue was subjected to flash column chromatography on silica gel (EtOAc/hexanes, 1:1). This afforded 3.7 g (90%) of **16d**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.53 (s, 9H), 2.80 (m, 4H), 3.44 (s, 2H), 3.82 (s, 2H), 5.89 (bs, 2H, −N***H***$_2$), 7.44 (m, 2H), 7.52 (dd, 1H), 7.79 (m, 4H).

Step 3. 2-(Ethoxyoxalylamino)-6-naphthalen-2-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (17d). To a solution of **16d** (0.6 g, 1.52 mmol) in THF (50 mL) was added imidazol-1-yloxoacetic acid *tert*-butyl ester (1.2 g, 6.08 mmol). The reaction mixture was stirred at room temperature for 62 h and evaporated. The residue was subjected to flash column chromatography on silica gel (EtOAc/hexanes, 1:4). This afforded 55 mg (7%) of **17d**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.57 (s, 9H), 1.60 (s, 9H), 2.80 (t, 2H), 2.89 (m, 2H), 3.63 (s, 2H), 3.84 (s, 2H), 7.44 (m, 2H), 7.52 (dd, 1H), 7.79 (m, 4H), 12.5 (s, 1H, −N***H***COCOO*t*-Bu).

Step 4. 6-Naphthalen-2-ylmethyl-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22d). Compound **17d** (55 mg, 0.11 mmol) was dissolved in a mixture of TFA and CH_2Cl_2 (1:2) (15 mL). The reaction was stirred at room temperature for 16 h and evaporated, and the residue was washed with ether and filtered off. After it was dried, this afforded 50 mg (91%) of **22d**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.06 (bs, 2H), 3.42 (bm, 2H), 4.23 (bs, 2H), 4.49 (bs, 2H), 7.51−7.60 (m, 3H), 7.90−7.99 (m, 4H), 12.3 (bs, 1H, N***H***COCOOH). Anal. ($C_{21}H_{18}N_2O_5S\cdot 0.8$TFA) C, H, N.

The following compounds were prepared by the method described for the preparation of compound **22d**.

6-(2-Naphthalen-1-ylethyl)-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22j). Step 1. 1-(2-Naphthalen-1-ylethyl)piperidin-4-one (15j). Yield 3.3 g (90%) of **15j**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.51 (t, 4H), 2.82−2.95 (m, 6H), 3.31 (m, 2H), 7.37 (m, 2H), 7.48 (m, 2H), 7.73 (d, 1H), 7.85 (d, 1H), 8.04 (d, 1H).

Step 2. 2-Amino-6-(2-naphthalen-1-ylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (16j). Yield 3.9 g (81%) of **16j**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.56 (s, 9H), 2.88 (m, 6H), 3.35 (m, 2H), 3.59 (s, 2H), 5.91 (s, 2H, −N***H***$_2$), 7.37 (m, 2H), 7.48 (m, 2H), 7.72 (d, 1H), 7.84 (d, 1H), 8.06 (d, 1H).

Step 3. 2-(*tert*-Butoxyoxalylamino)-6-(2-naphthalen-1-ylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (17j). Yield 3.5 g (68%) of **17j**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.59 (s, 9H), 1.62 (s, 9H), 2.92 (m, 6H), 3.38 (m, 2H), 3.76 (s, 2H), 7.39 (m, 2H), 7.49 (m, 2H), 7.74 (d, 1H), 7.85 (d, 1H), 8.06 (d, 1H), 12.5 (s, 1H, N***H***COCOO*t*-Bu).

Step 4. 6-(2-Naphthalen-1-ylethyl)-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22j). Yield 3.1 g (83%) of **22j**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.18 (bm, 3H), 3.55 (bm, 4H), 4.50−4.83 (bm, 3H), 7.47 (m, 2H), 7.58 (m, 2H), 7.97 (m, 1H), 8.14 (d, 1H), 12.3 (bs, 1H, N***H***COCOOH). Anal. ($C_{22}H_{20}N_2O_5S\cdot 1.75$TFA) C, H, N.

6-(2-Biphenyl-4-ylethyl)-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22k). Step 1. 1-(2-Biphenyl-4-ylethyl)piperidin-4-one (15k). Yield 1.4 g (81%) of **15k**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.50 (t, 4H), 2.74−2.92 (m, 8H), 7.30 (m, 3H), 7.42 (t, 2H), 7.53 (d, 2H), 7.57 (d, 2H).

Step 2. 2-Amino-6-(2-biphenyl-4-ylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (16k). Yield 1.4 g (75%) of **16k**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.54 (s, 9H), 2.82 (m, 6H), 2.91 (m, 2H), 3.53 (s, 2H), 5.89 (s, 2H, −N***H***$_2$), 7.30 (m, 3H), 7.42 (t, 2H), 7.51 (d, 2H), 7.57 (d, 2H).

Step 3. 6-(2-Biphenyl-4-ylethyl)-2-(*tert*-butoxyoxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (17k). Yield 0.8 g (62%) of **16k**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.59 (s, 9H), 1.62 (s, 9H), 2.84 (m, 4H), 2.93 (m, 4H), 3.70 (s, 2H), 7.30 (m, 3H), 7.42 (t, 2H), 7.51 (d, 2H), 7.57 (d, 2H), 12.45 (s, 1H, N***H***COCOO*t*-Bu).

Step 4. 6-(2-Biphenyl-4-ylethyl)-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22k). Yield 0.4 g (67%) of **22k**; mp >250 °C. ^{1}H NMR (DMSO-d_6): δ 3.14 (m, 3H), 3.50 (m, 2H), 3.60 (m, 2H), 4.53 (bs, 2H), 7.32−7.47 (m, 5H), 7.65 (d, 4H), 12.3 (bs, 1H, N***H***COCOOH). Anal. ($C_{24}H_{22}N_2O_5S\cdot 0.8$TFA) C, H, N.

6-(2-(2-Benzyloxyphenyl)ethyl)-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22m). Step 1. 1-(2-(2-Benzyloxyphenyl)ethyl)piperidin-4-one (15m). Yield 0.9 g (23%) of **15m**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.41 (t, 4H), 2.69−2.82 (m, 6H), 2.89 (m, 2H), 5.08 (s, 2H), 6.90 (t, 2H), 7.17 (m, 2H), 7.29−7.44 (m, 5H).

Step 2. 2-Amino-6-(2-(2-benzyloxyphenyl)ethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (16m). Yield 0.9 g (66%) of **16m**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.53 (s, 9H), 2.75 (m, 6H), 2.95 (m, 2H), 3.45 (s, 2H), 5.08 (s, 2H), 5.89 (s, 2H, −N***H***$_2$), 6.88 (m, 2H), 7.16 (m, 2H), 7.30−7.44 (m, 5H).

Step 3. 6-(2-(2-Benzyloxyphenyl)ethyl)-2-(*tert*-butoxyoxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (17m). Yield 0.25 g (24%) of **17m**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.58 (s, 9H), 1.62 (s, 9H), 2.78 (m, 4H), 2.89 (m, 2H), 2.96 (m, 2H), 3.60 (s, 2H), 5.08 (s, 2H), 6.90 (t, 2H), 7.17 (t, 2H), 7.32−7.44 (m, 5H), 12.5 (s, 1H, N***H***COCOO*t*-Bu).

Step 4. 6-(2-(2-Benzyloxyphenyl)ethyl)-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22m). Yield 15 mg (30%) of **22m**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.05 (m, 4H), 4.32 (bs, 2H), 5.14 (s, 2H), 6.91 (t, 1H), 7.07 (d, 1H), 7.23 (t, 2H), 7.28−7.39 (m, 3H), 7.46 (d, 2H). LC/MS $[M + H]^+$ *m*/*z* 482. Anal. ($C_{25}H_{24}N_2O_6S\cdot 0.4$TFA) C, H, N.

2-(Oxalylamino)-6-(3-phenylpropyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22q). Step 1. 1-(3-Phenylpropyl)piperidin-4-one (15q). Yield 3.75 g (~100%) of **15q**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (m, 2H), 2.50−2.80 (m, 8H), 2.97 (m, 4H), 7.13−7.22 (m, 3H), 7.27−7.31 (m, 2H).

Step 2. 2-Amino-6-(3-phenylpropyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (16q). Yield 4.19 g (65%) of **16q**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.53 (s, 9H), 1.89 (m, 2H), 2.53 (t, 2H), 2.67 (m, 4H), 2.79 (m, 2H), 3.40 (s, 2H), 5.98 (s, 2H, −N***H***$_2$), 7.19 (m, 3H), 7.27 (m, 2H).

Step 3. 2-(*tert*-Butoxyoxalylamino)-6-(3-phenylpropyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxylic Acid *tert*-Butyl Ester (17q). Yield 0.96 g (71%) of **17q**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.59 (s, 9H), 1.61 (s, 9H), 1.91 (m, 2H), 2.56 (t, 2H), 2.69 (t, 2H), 2.74 (t, 2H), 2.89 (m, 2H), 3.58 (s, 2H), 7.19 (m, 3H), 7.29 (m, 2H), 12.5 (s, 1H, N***H***COCOO*t*-Bu).

Step 4. 2-(Oxalylamino)-6-(3-phenylpropyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22q). Yield 190 mg (34%) of **22q**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.99−2.09 (m, 2H), 2.66 (t, J = 8.66 Hz, 2H), 3.11 (bs, 2H), 3.21−3.28 (m, 2H), 3.51 (bs, 2H), 4.46 (bs, 2H), 7.19−7.34 (m, 5H), 12.31 (bs, 1H, N***H***COCOOH). LC-MS (ESI): m/z 389.0 $[M + H]^+$. Anal. ($C_{19}H_{20}N_2O_5S$·1TFA) C, H, N.

6-(3-Methoxybenzyl)-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22c). Step 1. 2-Amino-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3,6-dicarboxylic Acid 6-*tert*-Butyl Ester 3-Ethyl Ester (19a). To a solution of 4-oxo-piperidine-1-carboxylic acid *tert*-butyl ester (**13b**) (24.6 g, 0.123 mol), ethyl cyanoacetate (15.4 g, 0.136 mmol), and sulfur (4.4 g, 0.136 mmol) in absolute ethanol (200 mL) was added morpholine (30 mL). The mixture was heated to 50 °C for 2.5 h and cooled, and to the residue was added ether. The precipitate was filtered off and washed with water (2 × 50 mL) and ether (50 mL). This afforded after drying 32.6 g (81%) of **19a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (t, 3H), 1.48 (s, 9H), 2.80 (m, 2H), 3.60 (t, 2H), 4.25 (q, 2H), 4.34 (s, 2H), 5.98 (bs, 2H, −NH_2).

Step 2. 2-(Ethoxyoxalylamino)-4,5,6,7-tetrahydrothieno-[2,3-c]pyridine-3,6-dicarboxylic Acid 6-*tert*-Butyl Ester 3-Ethyl Ester (20a). To a solution of **19a** (32.4 g, 0.099 mol) and TEA (20.7 mL, 0.15 mol) in THF (300 mL) was added dropwise a solution of ethyl oxalyl chloride (13.3 mL, 0.12 mol) in THF (130 mL) at 0 °C. The reaction mixture was stirred at room temperature for 16 h and evaporated. To the residue was added water (300 mL), and the mixture was extracted with EtOAc (3 × 100 mL). The organic phases were washed with water (100 mL) and brine (100 mL) and dried ($MgSO_4$), and the solvent was evaporated. This afforded 35.5 g (85%) of **20a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (q, 6H), 1.48 (s, 9H), 2.91 (m, 2H), 3.66 (t, 2H), 4.38 (q, 2H), 4.44 (q, 2H), 4.53 (s, 2H), 12.45 (s, 1H, N***H***COCOO*t*-Bu).

Step 3. 3-Ethoxycarbonyl-2-(ethoxyoxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-ium; Trifluoroacetate (11h). Compound **20a** (33.5 g, 0.079 mol) was dissolved in a mixture of TFA and CH_2Cl_2 (1:7) (350 mL). The reaction was stirred at room temperature for 16 h and evaporated, and the solid residue was washed with ether (200 mL) and filtered off. After it was dried, this afforded 33.6 g (97%) of **11h**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.34 (q, 6H), 3.03 (bm, 2H), 3.40 (t, 2H), 4.34 (m, 5H), 9.50 (bs, 2H, N***H***$_2^+$), 12.1 (bs, 1H, N***H***COCOOEt).

Step 4. 3-Ethoxycarbonyl-2-(ethoxyoxalylamino)-6-(3-methoxybenzyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine (21c). To a mixture of **11h** (1.0 g, 2.3 mmol) in acetone (35 mL) were added K_2CO_2 (1.3 g, 9.2 mmol), KI (20 mg), and 3-methoxybenzyl chloride (340 μL, 2.5 mmol). The reaction was stirred at reflux temperature for 16 h, cooled, and filtered, and the solvent was evaporated. The residue was subjected to flash column chromatography on silica gel (EtOAc/hexanes, 1:2). This afforded 0.5 g (49%) of **21c**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.39 (t, 3H), 1.43 (t, 3H), 2.77 (t, 2H), 2.91 (bt, 2H), 3.58 (s, 2H), 3.67 (s, 2H), 3.81 (s, 3H), 4.36 (q, 2H), 4.42 (q, 2H), 6.79 (dd, 1H), 6.91 m, 2H), 10.03 (t, 1H), 12.4 (s, 1H, N***H***COCOOEt).

Step 5. 6-(3-Methoxybenzyl)-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22c). To **21c** (0.5 g, 1.1 mmol) dissolved in a mixture of water and EtOH (1:1) (30 mL) was added 1 N NaOH (2.8 mL, 2.8 mmol). The reaction was stirred at room temperature for 16 h, evaporated to 1/2 volume, and acidified to pH 2 with 1 N HCl. The precipitate was filtered off, washed with ether, and dried. This afforded 230 mg (52%) of **22c**; mp 233−237 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.0 (bs, 2H), 3.28 (bs, 4H, partly obscured by water), 3.78 (s, 3H), 4.08 (bs, 1H), 4.21 (bs, 1H), 6.96 (dd, 1H), 7.03 (d, 1H), 7.07 (s, 1H), 7.33 (t, 1H), 12.2 (bs, 1H, N***H***COCOOH). Anal. ($C_{18}H_{18}N_2O_6S$·1H_2O) C, H, N.

The following compounds were prepared by the method described for the preparation of compound **22c**.

2-(Oxalylamino)-6-pyridin-2-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22e). Step 1. 2-(Oxalylamino)-6-pyridin-2-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid Ethyl Ester (21e). To a mixture of **11h** (1.5 g, 3.40 mmol), K_2CO_3 (2.4 g, 17.1 mmol), and KI (100 mg) in acetone (40 mL) was added 2-picolyl chloride hydrochloride (0.61 g, 3.7 mmol). The mixture was stirred at reflux temperature for 18 h, filtered, and evaporated. The residue was trituated with diethyl ether, and the solid was filtered off and purified by chromatography on silicagel (EtOAc/EtOH/TEA, 3:1:0.4) affording 650 mg (39%) of **21e** as a triethylammonium salt.

Step 2. 2-(Oxalylamino)-6-pyridin-2-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22e). To a solution of **21e** (650 mg, 1.40 mmol) in EtOH (15 mL) was added 1 N NaOH (4.1 mL, 4.1 mmol) followed by water (15 mL). The mixture was stirred at room temperature for 18 h and evaporated, and the residue was dissolved in water (20 mL) and washed with diethyl ether (2 × 10 mL). The aqueous phase was acidified with 1 N HCl to pH 1 and evaporated. The residue was suspended in a mixture of 2-propanol/water (1:1, 40 mL) and stirred for 1 h, the solid was filtered off, washed with 2-propanol (2 × 15 mL), and dried affording 181 mg (38%) of crude **22e**. The crude product was dissolved in a mixture of water (10 mL) and 5 N NaOH (10 mL) and washed with diethyl ether (2 × 10 mL). The aqueous phase was acidified to pH 3 with 1 N HCl, and the precipitate was filtered off, washed with water (3 × 20 mL), and dried affording 51 mg (11%) of **22e**; mp 238−244 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.15 (m, 2H), 3.53 (m, 2H), 4.44 (s, 2H), 4.59 (s, 2H), 7.48 (m, 1H), 7.66 (d, 1H), 7.94 (dt, 1H), 8.67 (d, 1H), 12.3 (bs, 1H, N***H***COCOOH), 13.65 (bs, 1H). Anal. ($C_{16}H_{15}N_3O_5S$·3H_2O) C, H, N.

2-(Oxalylamino)-6-pyridin-3-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22f). Step 1. 2-(Ethoxyoxalylamino)-6-pyridin-3-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid Ethyl Ester (21f). Yield 0.55 g (39%) of **21f**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, 3H), 1.41 (t, 3H), 2.78 (t, 2H), 2.92 (t, 2H), 3.60 (s, 2H), 3.71 (s, 2H), 4.37 (q, 2H), 4.44 (q, 2H), 7.28 (m, 1H), 7.72 (m, 1H), 8.52 (m, 1H), 8.58 (m, 1H), 12.43 (s, 1H, N***H***COCOOEt).

Step 2. 2-(Oxalylamino)-6-pyridin-3-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22f). Yield 64 mg (13%) of **22f**; mp 234−238 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.07 (s,2H), 3.35 (s, 2H), 4.18 (s, 2H), 4.37 (s, 2H), 7.48 (dd, 1H), 8.00 (d, 1H), 8.62 (d, 1H), 8.72 (s, 1H), 12.3 (bs, 1H, N***H***COCOOH). Anal. ($C_{16}H_{15}N_3O_5S$·1 HCl, 1/2H_2O) C, H, N.

2-(Oxalylamino)-6-pyridin-4-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22g). Step 1. 2-(Ethoxyoxalylamino)-6-pyridin-4-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid Ethyl Ester (21g). Yield 0.43 g (15%) of **21g**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (t, 3H), 1.41 (t, 3H), 2.77 (t, 2H), 2.93 (t, 2H), 3.61 (s, 2H), 3.70 (s, 2H), 4.37 (q, 2H), 4.43 (q, 2H), 7.30 (d, 2H), 8.55 (d, 2H), 12.4 (s, 1H, N***H***COCOOEt).

Step 2. 2-(Oxalylamino)-6-pyridin-4-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22g). Yield 104 mg (28%) of **22g**; mp 230−235 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.0 (s, 2H), 3.13 (s, 2H), 3.96 (s, 2H), 4.15 (s, 2H), 7.55 (d, 1H), 8.65 (d, 1H), 12.3 (bs, 1H, N***H***COCOOH). Anal. ($C_{16}H_{15}N_3O_5S$·1HCl, $1H_2O$) C, H, N.

2-(Oxalylamino)-6-quinolin-2-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22h). Step 1. 2-(Ethoxyoxalylamino)-6-quinolin-2-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid Ethyl Ester (21h). Yield 1.23 g (39%) of **21h**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, 3H), 1.43 (t, 3H), 2.88 (t, 2H), 2.95 (d, 2H), 3.73 (s, 2H), 4.05 (s, 2H), 4.38 (q, 2H), 4.44 (q, 2H), 7.52 (t, 1H), 7.69 (q, 2H), 7.81 (d, 1H), 8.08 (d, 1H), 8.13 (d, 1H), 12.4 (s, 1H, N***H***COCOOEt).

Step 2. 2-(Oxalylamino)-6-quinolin-2-ylmethyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22h). Yield 92 mg (11%) of **22h**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.95 (bs, 2H), 3.07 (bs, 2H), 3.92 (bs, 2H), 4.24 (bs, 2H), 7.60 (t, 1H), 7.66 (d, 1H), 7.76 (t, 1H), 7.99 (t, 1H), 8.37 (d, 1H), 12.3 (bs, 1H, N***H***COCOOH). Anal. ($C_{20}H_{17}N_3O_5S$·$1H_2O$) C, H, N.

2-(Oxalylamino)-6-(2-thiophen-3-ylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22n). Step 1. 2-Amino-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (18). To a solution of 4-oxo-piperidine, hydrochloride mono hydrate (**13a**) (20 g, 0.13 mol), *tert*-butyl cyanoacetate (20.2 g, 0.143 mmol), and sulfur (4.6 g, 0.143 mmol) in EtOH (350 mL) was added *N*-methyl morpholine (43 mL) followed by 4 Å molecular sieves (15 g). The mixture was heated to 50 °C for 7 h and cooled, and the precipitate was filtered off and washed with EtOH (50 mL). The solvent was evaporated, and the residue was subjected to flash column chromatography on silica gel (EtOAc (700 mL) the 4% TEA in EtOAc). This afforded 5.8 g (18%) of **18**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.56 (t, 9H), 2.70 (m, 2H), 3.05 (t, 2H), 3.77 (t, 2H), 5.91 (bs, 2H, −N***H***$_2$).

Step 2. 2-(*tert*-Butoxyoxalylamino)-6-(2-thiophen-3-ylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (17n). To a solution of **18** (0.6 g, 2.36 mmol) and 2-thiophen-3-ylethanol (0.3 g, 2.48 mmol) in propionitril (25 mL) was added (cyanomethyl)trimethylphosphonium iodine (0.69 g, 2.83 mmol) followed by DIPEA (0.53 mL, 3.07 mmol). The resulting mixture was heated at reflux for 2 h, cooled, and filtered. The residue was submitted to flash chromatography on silica gel (EtOAc/heptane, 1:3) to afford 0.5 g (58%) of 2-amino-6-(2-thiophen-3-ylethyl)-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid *tert*-butyl ester (**16n**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.53 (s, 9H), 2.78 (m, 3H), 2.90 (m, 2H), 3.50 (s, 2H), 5.89 (bs, 2H, −N***H***$_2$), 6.95 (m, 1H), 6.99 (m, 1H), 7.22 (m, 1H).

To a solution of the above *tert*-butyl ester (0.5 g, 1.37 mmol) in THF (40 mL) was added imidazol-1-yloxoacetic acid *tert*-butyl ester (0.53 g, 2.74 mmol). The reaction mixture was stirred at room temperature for 48 h and evaporated. The residue was subjected to flash column chromatography on silica gel (EtOAc/heptane, 1:3). This afforded 55 mg (81%) of **17n**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.60 (s, 9H), 1.62 (s, 9H), 2.83 (m, 4H), 2.92 (m, 4H), 3.66 (s, 2H), 6.96 (d, 1H), 7.00 (m, 1H), 7.23 (m, 1H), 12.5 (s, 1H, N**H**COCOO*t*-Bu).

Step 3. 2-(Oxalylamino)-6-(2-thiophen-3-ylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22n). To a solution of **17n** (32.4 g, 0.099 mol) and TEA (20.7 mL, 0.15 mol) in THF (300 mL) was added dropwise a solution of ethyl oxalyl chloride (13.3 mL, 0.12 mol) in THF (130 mL) at 0 °C. The reaction mixture was stirred at room temperature for 16 h and evaporated. To the residue was added water (300 mL), and the mixture was extracted with EtOAc (3 × 100 mL). The organic phases were washed with water (100 mL) and brine (100 mL) and dried ($MgSO_4$), and the solvent was evaporated. This afforded 190 mg (34%) of **22n**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.12 (m, 3H), 3.47 (bm, 6H), 4.47 (bs, 1H), 7.06 (d, 1H), 7.34 (s, 1H), 7.52 (m, 1H), 12.3 (bs, 1H, N***H***COCOOH). Anal. ($C_{16}H_{16}N_2O_5S_2$·0.6TFA) C, H, N.

The following compounds were prepared by the method described for the preparation of compound **22n**.

6-(2-(4-Benzyloxyphenyl)ethyl)-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22l). Step 1. 2-Amino-6-[2-(4-benzyloxyphenyl)ethyl]-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (16l). Yield 1.7 g (62%) of **16l**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.54 (s, 9H), 2.69−2.84 (m, 8H), 3.49 (s, 2H), 5.04 (s, 2H), 5.89 (bs, 2H, −N***H***$_2$), 6.90 (d, 2H), 7.13 (d, 2H), 7.29−7.43 (m, 5H).

Step 2. 6-(2-(4-Benzyloxyphenyl)ethyl)-2-(*tert*-butoxyoxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (17l). Yield 1.3 g (68%) of **17l**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.59 (s, 9H), 1.62 (s, 9H), 2.73−2.87 (m, 6H), 2.91 (m, 2H), 3.66 (s, 2H), 5.04 (s, 2H), 6.72 (d, 2H), 7.13 (d, 2H), 7.28−7.44 (m, 5H), 12,5 (s, 1H, N***H***CO-COO*t*-Bu).

Step 3. 6-(2-(4-Benzyloxyphenyl)ethyl)-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22l). Yield 240 mg (68%) of **22l**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.0 (m, 2H), 3.11 (bs, 2H), 3.4 (m, 2H), 3.55 (bs, 2H), 4.47 (bs, 2H), 5.07 (s, 2H), 6.97 (d, 2H), 7.20 (d, 2H), 7.3−7.43 (m, 5H), 12.3 (bs, 1H, N***H***COCOOH). Anal. ($C_{25}H_{24}N_2O_6S$·0.6TFA) C, H, N.

2-(Oxalylamino)-6-(2-pyridin-2-ylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22o). Step 1. 2-Amino-6-(2-pyridin-2-ylethyl)-4,5,6,7-tetrahydrothieno-[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (16o). Yield 0.5 g (59%) of **16o**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.52 (s, 9H), 2.80 (s, 4H), 2.92 (m, 2H), 3.05 (m, 2H), 3.52 (s, 2H), 5.91 (s, 2H, −N***H***$_2$), 7.11 (dd, 1H), 7.19 (d, 1H), 7.59 (dt, 1H), 8.51 (d, 1H).

Step 2. 2-(*tert*-Butoxyoxalylamino)-6-(2-pyridin-2-ylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (17o). Yield 0.6 g (88%) of **17o**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.62 (s, 9H), 1.64 (s, 9H), 2.85−3.01 (m, 6H), 3.07 (m, 2H), 3.68 (s, 2H), 7.11 (dd, 1H), 7.20 (d, 1H), 7.59 (dt, 1H), 8.53 (d, 1H), 12,5 (s, 1H, N***H***COCOO*t*-Bu).

Step 3. 2-(Oxalylamino)-6-(2-pyridin-2-ylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22o). Yield 450 mg (75%) of **22o**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.14 (bs, 2H), 3.27 (t, 2H), 3.63 (m, 4H), 4.55 (bs, 2H), 7.33 (dd, 1H), 7.38 (d, 1H), 7.80 (dt, 1H), 8.53 (d, 1H), 12.3 (bs, 1H, N***H***COCOOH). Anal. ($C_{17}H_{17}N_3O_5S$·1.5TFA) C, H, N.

2-(Oxalylamino)-6-(2-pyridin-4-ylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22p). Step 1. 2-Amino-6-(2-pyridin-4-ylethyl)-4,5,6,7-tetrahydrothieno-[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (16p). Yield 0.6 g (88%) of **16p**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.54 (s, 9H), 2.74−2.89 (m, 8H), 3.50 (s, 2H), 5.91 (s, 2H, −N***H***$_2$), 7.15 (d, 2H), 8.49 (d, 2H).

Step 2. 2-(*tert*-Butoxyoxalylamino)-6-(2-pyridin-4-ylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (17p). Yield 0.15 g (74%) of **17p**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.59 (s, 9H), 1.61 (s, 9H), 2.78−2.90 (m, 8H), 3.66 (s, 2H), 7.15 (d, 2H), 8.49 (d, 2H), 12.5 (s, 1H, N***H***COCOO*t*-Bu).

Step 3. 2-(Oxalylamino)-6-(2-pyridin-4-ylethyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22p). Yield 90 mg (60%) of **22p**; mp >250 °C. ^{1}H NMR (DMSO-d_6): δ 3.15 (m, 4H), 3.53 (m, 4H), 4.50 (bs, 2H), 7.48 (d, 2H), 8.60 (d, 2H), 12.3 (bs, 1H, N***H***COCOOH). Anal. ($C_{17}H_{17}N_3O_5S$·1.4TFA) C, H, N.

6-(3,3-Diphenylpropyl)-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22r). Step 1. 2-Amino-6-(3,3-diphenylpropyl)-4,5,6,7-tetrahydrothieno-[2,3-c]pyridine-3-carboxylic Acid *tert*-Butyl Ester (16r). Yield 0.6 g (57%) of **16r**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.52 (s, 9H), 2.31 (m, 2H), 2.43 (m, 2H), 2.65 (t, 2H), 2.76 (m, 2H), 3.37 (s, 2H), 4.04 (t, 1H), 5.87 (s, 2H, −N***H***$_2$), 7.16 (m, 2H), 7.25 (m, 8H).

Step 2. 2-(*tert*-Butoxyoxalylamino)-6-(3,3-diphenylpropyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxy-

lic Acid *tert*-Butyl Ester (17r). Yield 0.2 g (26%) of **17r**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61 (s, 9H), 1.63 (s, 9H), 2.32 (m, 2H), 2.46 (m, 2H), 2.69 (t, 2H), 2.85 (m, 2H), 3.54 (s, 2H), 4.04 (t, 1H), 7.17 (m, 2H), 7.25 (m, 8H), 12.45 (s, 1H, N***H***COCOO*t*-Bu).

Step 3. 6-(3,3-Diphenylpropyl)-2-(oxalylamino)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic Acid (22r). Yield 100 mg (50%) of **22r**; mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.54 (m, 1H), 3.06 (m, 3H), 3.50 (bm, 5H), 4.05 (t, 1H), 4.42 (bs, 1H), 7.18 (t, 2H), 7.26−7.34 (m, 8H), 12.25 (bs, 1H, N***H***COCOOH). Anal. ($C_{25}H_{24}N_2O_5S\cdot 0.6$TFA) C, H, N.

Supporting Information Available: Cocrystallizing of PTP1B with compounds **8e** and **22i**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Fischer, E. H.; Charbonneau, H.; Tonks, N. K. Protein tyrosine phosphatases: A diverse family of intracellular and transmembrane enzymes. *Science* **1991**, *13*, 1497−1506.

(2) Hunter, T. Signaling − 2000 and beyond. *Cell* **2000**, *100*, 113−127.

(3) Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Lee Loy, A.; Normandin, D.; Cheng, A.; Himmshagen, 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.

(4) Andersen, H. S.; Iversen, L. F.; Jeppesen, C. B.; Branner, S.; Norris, K.; Rasmussen, H. B.; Møller, K. B.; Møller, N. P. H. 2-(Oxalyl-amino)-benzoic acid is a general, competitive inhibitor of protein-tyrosine phosphatases. *J. Biol. Chem*. **2000**, *275*, 7101−7108.

(5) McCormack, J. C.; Iversen, L. F.; Andersen, H. S.; Møller, N. P. H. Protein-tyrosine phosphatases (PTPs) as drug targets: Inhibitors of PTP-1B for the treatment of diabetes. *Curr. Opin. Drug Discovery Dev.* **2000**, *3*, 527−540.

(6) Zhang, Z. Y.; Wang, Y.; Wu, L.; Fauman, E. B.; Stuckey, J. A.; Schubert, H. L.; Saper, M. A.; Dixon, J. E. *Biochemistry* **1994**, *33*, 15266−15270.

(7) Denu, J. M.; Dixon, J. E. Protein tyrosine phosphatases: mechanisms of catalysis and regulation. *Curr. Opin. Chem. Biol.* **1998**, *2*, 633−641.

(8) Bleasdale, J. E.; Ogg, D.; Palazuk, B. J.; Jacob, C. S.; Swanson, M. L.; Wang, X.-Y.; Thompson, D. P.; Conradi, R. A.; Mathews, R. W.; Laborde, A. L.; Stuchly, C. W.; Heijbel, A.; Bergdahl, K.; Bannow, C. A.; Smith, C. W.; Svensson, C.; Liljebris, C.; Schostarez, H. J.; May, P. D.; Stevens, F. C.; Larsen, S. D. Small Molecule Peptidomimetics Containing a Novel Phosphotyrosine Bioisostere Inhibit Protein Tyrosine Phosphatase 1B and Augment Insulin Action. *Biochemistry* **2001**, *40*, 5642−5654.

(9) Andersen, J. N. Personal communication.

(10) Andersen, J. N.; Mortensen, O. H.; Peters, G. H.; Drake, P. G.; Iversen, L. F.; Olsen, O. H.; Jansen, P. G.; Andersen, H. S.; Tonks, N. K.; Møller, N. P. H. Structural and Evolutionary Relationships among Protein Tyrosine Phosphatase Domains. *Mol. Cell. Biol.* **2001** *21* (21), 7117−7136.

(11) 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. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13420−13425.

(12) Pannifer, A. D. B.; Flint, A. J.; Tonks, N. K.; Barford, D. Visualization of the cysteinyl-phosphate intermediate of a protein tyrosine phosphatases by X-ray crystallography. *J. Biol. Chem*. **1998**, *273*, 10454−10462.

(13) Sarmiento, M.; Zhao, Y.; Gordon, S. J.; Zhang, Z. Y. Molecular basis for substrate specificity of protein-tyrosine phosphatase 1B. *J. Biol. Chem*. **1998**, *273*, 26368−26374.

(14) Iversen, L. F.; Andersen, H. S.; Branner, S.; Mortensen, S. B.; Peters, G.; Norris, K.; Olsen, O. H.; Lundt, B. F.; Ripka, W.; Møller, K. B.; Møller, N. P. H. Structure-based Design of a Low Molecular Weight, Non-phosphorus, Non-peptide and Highly Selective Inhibitor of Protein-Tyrosine Phosphatase 1B. *J. Biol. Chem*. **2000**, *275*, 10300−10307.

(15) Hartmann, H.; Liebscher, J. A simple method for the synthesis of 5-aryl-3-amino-2-alkoxycarbonylthiophenes. *Synthesis* **1984**, 275−276.

(16) Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. Predicting receptor−ligand interactions by an incremental construction algorithm. *J. Mol. Biol.* **1996**, *261*, 470−489.

(17) Gewald, K.; Schinke, E.; Böttcher, H. 2-Amino-thiophene aus methylenaktiven Nitrile, carbonylverbindiungen und Schwefel. *Chem. Ber.* **1966**, *99*, 94−100.

(18) Nimitz, J. S.; Mosher, H. S. A new synthesis of α-keto esters and acids. *J. Org. Chem*. **1981**, *46*, 211−213.

(19) Peters, G. H.; Iversen, L. F.; Branner, S.; Andersen, H. S.; Mortensen, S. B.; Olsen, O. H.; Møller, K. B.; Møller, N. P. H. Residue 259 Is a Key Determinant of Substrate Specificity of Protein-tyrosine Phosphatases 1B and α. *J. Biol. Chem*. **2001**, *275*, 18201−18209.

(20) Iversen, L. F.; Andersen, H. S.; Møller, K. B.; Olsen, O. H.; Peters, G. H.; Branner, S.; Mortensen, S. B.; Hansen, T. K.; Lau, J.; Ge, Yu.; Holsworth, D. D.; Newman, M. J.; Møller, N. P. H. Steric Hindrance as a Basic for Structure-based Design of Selective Inhibitor of Protein-Tyrosine Phosphatases. *Biochemistry* **2001**, *40*, 14812−14820.

(21) Zaragoza, F.; Stephensen, H. (Cyanomethyl)trialkylphosphonium Iodides: Efficient Reagents for the Intermolecular Alkylation of Amines with Alcohols in Solution and on Solid Phase. *J. Org. Chem*. **2001**, *66*, 2518−2521.

(22) Jia, Z. C.; Barford, D.; Flint, A. J.; Tonks, N. K. Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatases 1B. *Science* **1995**, *268*, 1754−1758.

(23) Burke, T. R., Jr.; Ye, B.; Yan, X.; Wang, S.; Jia, Z.; Chen, L.; Zhang, Z.-Y.; Barford, D. Small Molecule Interactions with Protein-Tyrosine Phosphatase PTP1B and Their Use in Inhibitor Design. *Biochemistry* **1996**, *35*, 15989−15996.

(24) Groves, M. R.; Yao, Z.-J.; Roller, P. P.; Burke, T. R.; Barford, D. Structural basis for inhibition of the protein tyrosine phosphatases 1B by phosphotyrosine peptide mimetics. *Biochemistry* **1998**, *37*, 17773−17783.

(25) Denu, J. M.; Lohse, D. L.; Vijayalakshmi, J.; Saper, M. A.; Dixon, J. E. Visualization of intermediate and transition-state structures in protein-tyrosine phosphatase catalysis. *Biochemistry* **1996**, *35*, 2493−2498.

(26) Salmeen, A.; Andersen, J. N.; Myers, M. P.; Tonks, N. K.; Barford, D. Molecular basis for the dephosphorylation of the active segment of the insulin receptor by protein tyrosine phosphatase 1B. *Mol. Cell* **2000**, *6*, 1401−1412.

(27) Wrobel, J.; Sredy, J.; Moxham, C.; Dietrich, A.; Li, Z.; Sawicki, D. R.; Seestaller, L.; Wu, L.; Katz, A.; Sullivan, D.; Tio, C.; Zhang, Z.-Z. PTP1B Inhibition and Antihyperglycemic Activity in the ob/ob Mouse Model of Novel 11-Aryl-benzo[*b*]naphtho[2,3-*d*]-furans and 11-Arylbenzo[*b*]naphtho[2,3-*d*]thiophenes. *J. Med. Chem*. **1999**, *42*, 3199−3202.

(28) Malamas, M. S.; J.; Sredy, J.; Moxham, C.; Katz, A.; Xu, W.; McDevitt, R.; Adebayo, F. O.; Sawicki, D. R.; Seestaller, L.; Sullivan, D.; Taylor, J. R. Novel Benzofuran and Benzothiophene Biphenyls as Inhibitors of Protein Tyrosine Phosphatase 1B with Antihyperglycemic Properties. *J. Med. Chem*. **2000**, *43*, 1293−1310.

(29) Burke, T. R.; Yao, Z.-J.; He, Z.; Milne, G. W. A.; Li, W.; Zhang, Z.-Y.; Voigt, J. H. Enantioselective Synthesis of Nonphosphorus-Containing Phosphotyrosyl Mimetics and Their Use in the Preparation of Tyrosine Phosphatase Inhibitory Peptides. *Tetrahedron* **1998**, *54*, 9981−9994.

(30) Berridge, M. V.; Tan, A. S. IL-3 facilitates glucose transport in a myeloid cell line by regulating the affinity of the glucose transporter for glucose: Involvement of protein phosphorylation in transporter activation. *Biochem. J.* **1995**, *305*, 843−851.

(31) Adson, A.; Raub, T. J.; Burton, P. S.; Barshun, C. L.; Hilgers, A. R.; Audus, K. L.; Ho, N. F. H. Quantitative approaches to delineate paracellular diffusion in cultured epithelial cell monolayers. *J. Pharm. Sci.* **1994**, *83*, 1529−1536.

(32) Taub, M. E.; Kristensen, L.; Frokjaer, S. Optimized conditions for MDCK permeability and turbidimetric solubility studies using compounds representative of BCS classes I−IV. *Eur. J. Pharm. Sci.* **2002**, *15*, 331−340.

(33) Showalter, H. D. H.; Sun, L.; Sercel, A. D.; Winters, R. T.; Denny, W. A.; Palmer, B. D. Concise Synthesis of the Novel 1*H*-Pyrrolo-[3,2-g]quinazoline Ring System and its [2,3-f] Angular Isomer. *J. Org. Chem*. **1996**, *61*, 1155−1158.

(34) Leonard, N. J.; Johnson, C. R. Periodate Oxidation of Sulfides to Sulfoxides. *J. Org. Chem*. **1962**, *27*, 282−284.

(35) Rule, N. G.; Detty, M. R.; Kaeding, J. E.; Sinicropi, J. A. Synthesis of 4*H*-Thiopyran-4-one 1,1-Dioxides as Precursors to Sulfone-Containing Analogues of Tetracyanoquinodimethane. *J. Org. Chem*. **1995**, *60*, 1665−1673.

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