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Bicyclic and tricyclic thiophenes as protein tyrosine phosphatase 1B inhibitors

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Abstract—A novel pyridothiophene inhibitor of PTP1B was discovered by rational screening of phosphotyrosine mimics at high micromolar concentrations. The potency of this lead compound has been improved significantly by medicinal chemistry guided by X-ray crystallography and molecular modeling. Excellent consistency has been observed between structure–activity relationships and structural information from PTP1B-inhibitor complexes.

1. Introduction

Protein tyrosine phosphatase 1B (PTP1B) has been shown to be a negative regulator in the insulin and leptin receptor pathways.[1–3] Two independent studies of PTP1B-deficient mice have revealed phenotypes of enhanced insulin sensitivity, improved glycemic control, and resistance to high-fat diet induced obesity.[4,5] Furthermore, treatment of diabetic mice with PTP1B antisense oligonucleotides reduced the expression level of the enzyme and subsequently normalized blood glucose and improved insulin sensitivity.[6,7] Thus, PTP1B has become an attractive therapeutic target for the treatment of type 2 diabetes and obesity. A number of companies and academic institutions have small molecule programs targeting this enzyme.[8–14]

Our PTP1B small molecule inhibitor program started with a high-throughput screening (HTS) campaign. About 6000 hits were identified with more than 40% inhibition at a concentration of 20 μg/mL. However, none of the selected HTS hits were real inhibitors of PTP1B upon cross-validation with detailed enzymology, NMR-binding studies, and X-ray crystallography. Such a high frequency of false positives could be due to the low pK_a and thus high reactivity of Cys215 at the enzyme active site.[15,16] Inhibition of PTP1B enzymatic activity via oxidation of the cysteine residue has been reported before.[17–19]

The lead compound **1** was discovered by screening several hundred compounds at high micromolar concentration for novel phosphotyrosine mimetics (Fig. 1). Compound **1** was a reversible and competitive inhibitor of PTP1B with a K_i of 230 μM at pH 7.4. Inhibition of the enzyme by this compound was independent of pH (K_i = 200 μM at pH 5.5).[20,21]

Binding of **1** to the enzyme active site was further confirmed by X-ray crystallography (Fig. 2). Upon binding of **1**, the WPD loop of PTP1B adopted a closed confor-

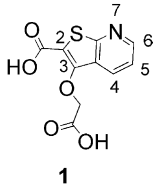

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Figure 1. Compound **1**, a reversible inhibitor of PTP1B.

Keywords: Protein tyrosine phosphatase 1B; Thiophene; Structure-based design.
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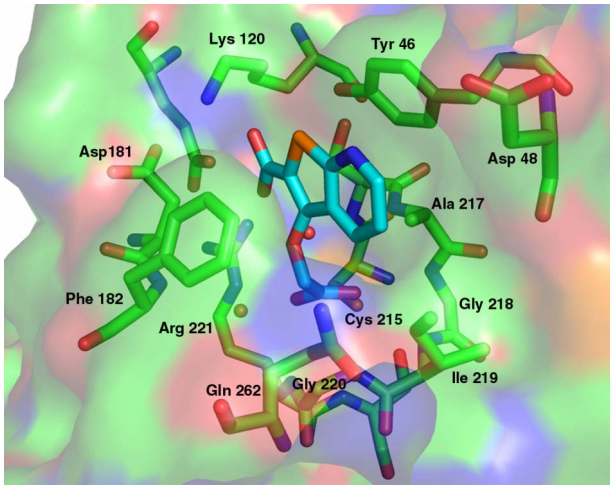


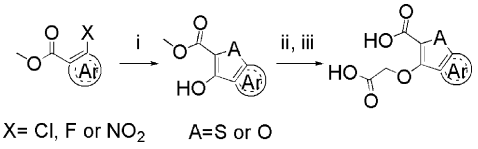
Figure 2. X-ray crystal structure of inhibitor **1** bound to PTP1B.

mation. The thiophene ring was sandwiched between Phe182 and Tyr46, which provided π-interactions and mimicked the phenyl group of phosphotyrosine (*p*-Tyr). The acidic side chain at the 3-position was buried deep down in the enzyme active site, mimicking the phosphate group of *p*-Tyr. The carboxyl group formed a salt bridge with Arg221 and multiple hydrogen bonds with the backbone amides of Gly218, Ile219, and Gly220 at the bottom of the active site. The ether oxygen formed water mediated hydrogen bonds with Ala217 and Arg221. The carboxyl group at the 2-position interacted with Lys120 via a salt bridge. The 5-position of **1** packed tightly against the side chain of Ile219. Because of these multiple interactions, the inhibitor positioned itself nicely at the enzyme active site.

Though the potency of **1** was weak, the availability of structural information provided guidance for further optimization. We report here our medicinal chemistry effort in significantly improving the potency of **1**. The structural–activity relationship will be discussed in conjunction with X-ray structural information and molecular modeling.

2. Chemistry

Synthesis of various bicyclic scaffolds was carried out following the general synthetic steps in Scheme 1.[22,23] As an example, reaction of ethyl 2-chloronicotinate and sodium methoxy-carbonylmethoxide afforded 3-hydroxyfuro[2,3-*b*]pyridine-2-carboxylate.[24] Subsequent alkylation with *tert*-butylbromoacetate followed by



Scheme 1. Reagents: (i) NaH or K_2CO_3, methyl 2-mercaptoacetate or methyl 2-hydroxyacetate, DMF; (ii) ethyl bromoacetate or *tert*-butyl 2-bromoacetate, K_2CO_3, DMF (iii) LiOH, THF/H_2O.

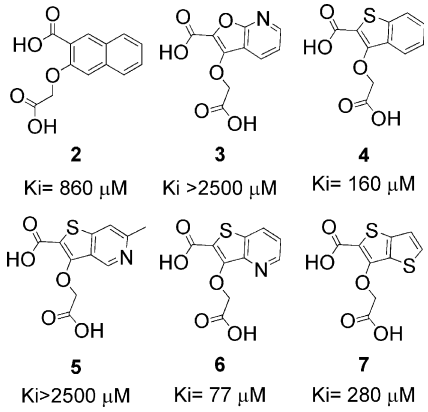


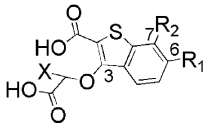
Figure 3. Variation of the bicyclic scaffolds on compound **1**.

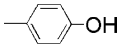
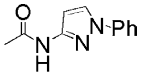
hydrolysis with LiOH provided compound **3** (Fig. 3). Compounds **4**–**7** were prepared in a similar manner. The ester precursor of **2** was prepared by alkylation of methyl 3-hydroxy-2-naphthoate with ethyl bromoacetate, followed by hydrolysis to give compound **2**.

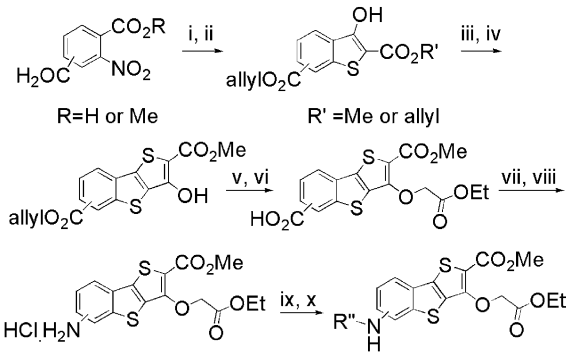
Further derivatization of benzothiophenes (compounds **8**–**17** in Table 1) was carried out according to the steps in Scheme 1, followed by straightforward functional group manipulations such as nitro reduction, acylation, reductive amination, amide formation, and cross-coupling reactions.[25–27]

Synthesis of thienobenzothiophene analogs was carried out following the steps in Scheme 2. 4-(Methoxycarbonyl)-3-nitrobenzoic acid or 2-nitro-isophthalic acid was treated with allyl bromide to protect the carboxylic acid moiety. The resulting allyl ester was treated with methyl 2-mercaptoacetate to effect formation of the second ring. Converting this bicycle to the corresponding triflate and

Table 1. Structure–activity relationship of the 3, 6, and 7 positions of benzothiophenes



Compound	X	R_1	R_2	PTP1B K_i (μM)
8	H	Cl	H	61
9	Me	Cl	H	>2500
10	F	Cl	H	52
11	H	Br	H	42
12	H		H	128
13	H		H	26
14	H		H	30
15	H		H	20
16	H	H	Cl	119
17	H	H	Me	37



Scheme 2. Reagents: (i) allyl bromide, K_2CO_3, DMF; (ii) methyl 2-mercaptoacetate, LiOH, DMF; (iii) Tf_2O, TEA, $CDCl_3$; (iv) methyl 2-mercaptoacetate, NaH, DMF; (v) ethyl bromoacetate, K_2CO_3, DMF; (vi) $Pd(PPh_3)_4$, morpholine, THF; (vii) DPPA, TEA, toluene, *t*-buOH; (viii) HCl, EtOAc, MeOH; (ix) aldehyde/ketone, $NaB(OAc)_3H$, DCE; (x) LiOH, THF/H_2O.

repeating treatment with methyl 2-mercaptoacetate formed the second thiophene ring. This was followed by alkylation with ethyl bromoacetate and deprotection of the allyl ester. Subsequent Curtius rearrangement of the free acid gave the entire thienobenzothiophene backbone, with an aniline nitrogen available for subsequent functionalization such as reductive amination.

3. Results and discussion

Optimization of **1** started with varying the pyridothiophene scaffold. Thiophene as the first ring of the bicyclic scaffold was crucial for better inhibitory activity against PTP1B (Fig. 3). A simple replacement of the sulfur atom with an oxygen atom resulted in significant loss of potency (**1** vs **3**). Expansion of the 5-membered thiophene ring to a 6-membered benzene ring also reduced activity significantly (**2** vs **4**).[28–30] On the contrary, the second ring was less sensitive to modifications. Moving the nitrogen from the 7-position (**1**) to the 4-position (**6**) or replacing of the nitrogen with a carbon (**4**) was allowed, but nitrogen at the 5-position (**5**) was not tolerated. Contraction of the 6-membered ring to a 5-membered thiophene ring was also tolerated.

The incompatibility of a polar nitrogen atom at the 5-position was consistent with the structural information in Figure 2, because this position interacted closely with the hydrophobic side chain of Ile219. Furthermore, molecular modeling suggested that this position would not allow further substitution.

Benzothiophenes were selected for further derivatization at the 3, 6, and 7-positions, since benzothiophene (**4**) offered a slightly better activity than pyridothiophene (**1**) and more flexibility in synthesis. The carboxymethoxy side chain at the 3-position appeared to be optimal. A fluorine was tolerated at the methylene carbon of the side chain (**8** vs **10**), but a simple methyl group at the same position resulted in significant loss of activity (**8** vs **9**). This likely reflects the energy cost of placing a hydrophobic group into the polar environment of the PTP1B active site (Fig. 2). Modeling suggests that a di-fluoro methylene or the addition of a methyl alcohol to the methylene carbon could be tolerated at this position; in the latter case, a bound water would be displaced.

Substitution at the 6-position offered the first hint at potency improvement. A simple chloro (**8**) or bromo (**11**) group provided a 3- to 4-fold increase in inhibitory activity. Electron-rich aromatic groups were favorable at this position (**12** vs **13** or **14**), as well as a carbonyl group (i.e., **15**). Though all these substitutions offered modest improvement in potency ranging from 2- to 8-fold versus that of **4**, the structure–activity relationship was relatively flat with K_i values ranging from 20 to 150 μM.

To our surprise, a simple methyl group at the 7-position of the benzothiophene yielded a compound (**17**) with a K_i of 37 μM, a 4-fold improvement over that of **4**. A model of **17** docked into the PTP1B active site indicates that the methyl group has some van der Waals interaction with the side chain of Tyr46 (Fig. 4). Since substitutions at either the 6- or 7-position provided modest improvement in potency, combining 6,7-di-substitution in the form of a benzo-fusion provided a tricyclic thiophene compound **18** (Fig. 5). The effect of this di-substitution turned out to be additive and compound **18** had a K_i of 11 μM, a 15-fold improvement over that of **4**. Two other tricyclic thiophene analogs (**19** and **20**) were prepared varying the middle ring. Consistent with the

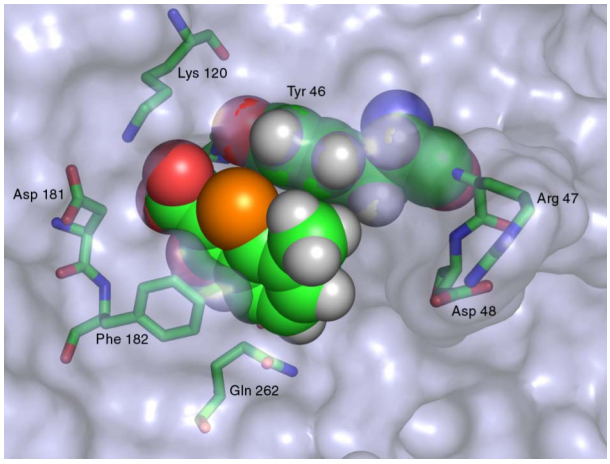


Figure 4. A model of compound **17** docked into the PTP1B active site. Compound **17** and Tyr46 are shown in a van der Waals sphere representation.

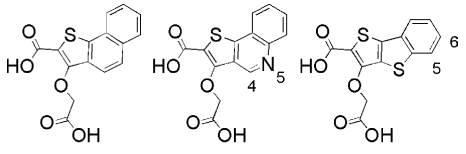


Figure 5. Tricyclic thiophene PTP1B inhibitors.

SAR of the bicyclic thiophenes, a thiophene ring was tolerated (**20**), while a pyridine nitrogen at the 5-position (**19**) resulted in significant loss of potency. The thienobenzothiophene template of **20** was chosen over that of **18** to be the new backbone of our chemical series due to greater novelty and synthetic flexibility.

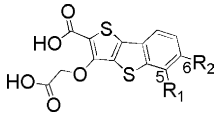
To further improve the potency and selectivity of **20**, our approach was to extend the inhibitor toward the second phosphotyrosine-binding site.[31,32] Molecular modeling suggested that substitution at the 5- or the 6-position could lead to the desired secondary interactions with the enzyme. Therefore, a number of compounds with each substitution pattern were synthesized and assayed.

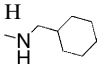
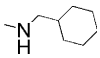
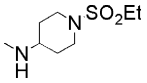
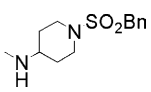
Table 2 shows representative examples of 5- or 6-substituted thienobenzothiophene compounds and their activity in our in vitro enzymatic assay. As predicted from molecular modeling, both the 5- and 6-positions offered opportunity for potency improvement. A cyclohexylmethylamino group at either position yielded compounds with sub-micromolar potency (**23** and **24**), which was an order of magnitude better than that of **20**. In general, substitution at the 6-position was more favorable than that at the 5-position (**22** vs **21**, **24** vs **23**, and **26** vs **25**), suggesting that the former position might provide a better trajectory toward the second phosphotyrosine-binding site. Thus, further extension of the molecule was derived from the 6-position.

Modeling suggested that the potency improvement from the cyclohexyl (**26**) or cyclohexylmethyl (**24**) group was mainly due to their van der Waals interaction with the Met258 side chain. Thus, it was not surprising that polar functionality such as oxygen (**27**) on the cyclohexyl ring resulted in slight loss in activity. Nevertheless, a piperdine group was introduced as a functional handle to gain further access to the second phosphotyrosine-binding site. A variety of sulfonamide derivatives were synthesized (e.g., **28** and **29**). Compound **29** turned out to be one of the most potent analogs in this chemical series, with a K_i of 370 nm. The X-ray co-crystal structures of **29** and PTP1B gave insight into the interactions that resulted in this dramatic increase in activity, as well as into ways that these interactions can be further optimized. As shown in Figure 6, the acid moieties of **29** bind to the active site in a similar binding mode as the bicyclic thiophene compounds (Fig. 2). In addition, one of the sulfonamide oxygens hydrogen bonds to the backbone nitrogen of Gly 259 and the other enters into interactions with Arg24 and Arg254 through bridging water molecules. These interactions overcome any unfavorable effect of the proximity of the piperidine nitrogen to the hydrophobic Met258 side chain. However, the benzyl moiety is pointing out into solvent. This shows that, presumably, further optimization of the side chain and/or the main scaffold, and therefore increased potency, is possible.

In general, the thienobenzothiophene analogs were very selective against other protein tyrosine phosphatases such as CD45 and LAR (~1000-fold), except the highly homologous TCPTP (Table 3).[33,34] Nevertheless, extending the inhibitor from the active site into the second phosphotyrosine-binding site did shift the relative selectivity of PTP1B vs TCPTP (**20** vs **29**) slightly. Compound **20**, only binding to the active site, favored TCPTP by about 2-fold. Compound **29**, spanning the active and the second phosphotyrosine-binding site, was equally potent against both enzymes.

Table 2. In vitro activity of 5- and 6-substituted thienobenzothiophenes



Compound	R_1	R_2	PTP1B K_i (μM)
21	Cl	H	10
22	H	Cl	3.5
23		H	0.92
24	H		0.68
25		H	1.7
26	H		0.74
27	H		2.4
28	H		1.6
29	H		0.37

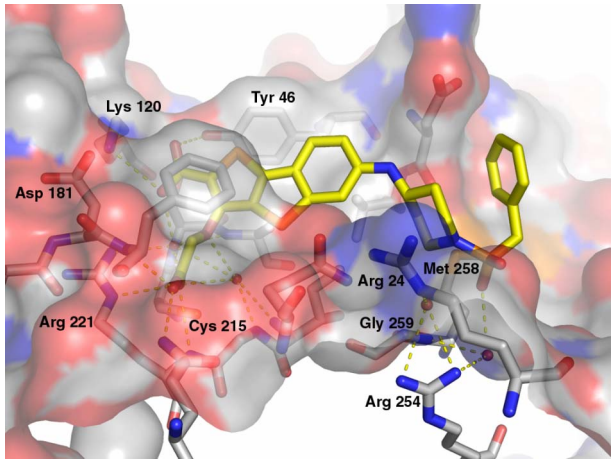


Figure 6. Co-crystal structure of **29** and PTP1B.

Table 3. Inhibitory activity of compounds **20** and **29** against various protein tyrosine phosphatases

Compound	K_i (μM)			
	PTP1B	CD45	LAR	TCPTP
20	9.2	>1250	1100	4.1
29	0.37	325	>500	0.38

4. Conclusion

In summary, a novel pyridothiophene lead was identified via screening of phosphotyrosine mimetics at high micromolar concentrations. Starting with this weak but well-characterized lead, potency has been improved almost three orders of magnitude. This improvement was achieved via medicinal chemistry guided by X-ray crystallography and molecular modeling. More importantly, the consistency of structure–activity relationship data with crystallographic information confirms that we can utilize structure-based drug design for efficiently effecting further optimization. This serves as a good starting point for developing low nanomolar PTP1B inhibitors by further optimizing the occupancy of the active site and the second phosphotyrosine-binding site. Effort in that regard will be reported in due course.

5. Experimental

5.1. General

Commercial reagents and solvents were used as received without further purification. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer. LC–MS data were collected using a Micromass LCT mass spectrometer with electrospray ionization in conjunction with a Waters 2795 LC system. Liquid chromatography (LC–MS) was performed using a Phenomenex C18 column (Mercury MS Luna 5μ C18(2), 20 × 2 mm) with mobile phase of 0.1% formic acid in H_2O (A) and 0.1% formic acid in CH_3CN (B) and a gradient of 15–100% B in 3 min followed by 1.5 min at 100% B. HRMS data was recorded on a Bruker APEXIII-7T FTMS spectrometer with electrospray ionization.

5.2. Synthesis

5.2.1. 3-(Carboxymethoxy)thieno[2,3-*b*]pyridine-2-carboxylic acid (1). Methyl 3-hydroxythieno[2,3-*b*]pyridine-2-carboxylate (**1a**) was prepared following literature procedures.[35] To a 5 mL DMF solution of **1a** (100 mg, 0.48 mmol) was added potassium carbonate (132 mg, 0.96 mmol) followed by ethyl bromoacetate (96 mg, 0.58 mmol). The reaction mixture was stirred at 60 °C for 4 h, then cooled to room temperature and poured into water (50 mL). A white precipitate emerged, and the solid was filtered, washed with water, and dried to afford methyl 3-(2-ethoxy-2-oxoethoxy)thieno[2,3-*b*]pyridine-2-carboxylate (**1b**; 115 mg, 81%). Compound **1b** (110 mg, 0.37 mmol) was dissolved in 1 mL THF and 2 mL of 1 N LiOH. The mixture was stirred at room temperature for 16 h. The solution was acidified with 1 N HCl slowly and a white precipitate was formed. The solid was filtered, washed with water, and dried to give compound **1** (73 mg, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.03 (s, 2H) 7.55 (dd, J = 8.34, 4.80 Hz, 1H) 8.36 (d, J = 8.34 Hz, 1H) 8.75 (d, J = 4.80 Hz, 1H); HRMS: calcd for $C_{10}H_7NO_5S$ + H+, 254.01177; found (ESI-FTMS, $[M+H]^{1+}$), 254.0116.

5.2.2. 3-(Carboxymethoxy)-2-naphthoic acid (2). Methyl 3-hydroxy-2-naphthoate (500 mg, 2.47 mmol), ethyl bromoacetate (619 mg, 3.71 mmol), and potassium carbonate (682 mg, 4.94 mmol) were suspended in 5 mL DMF. The mixture was stirred at 60 °C for 16 h. EtOAc (100 mL) was then added and the organic layer was washed with water, brine, and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure to afford methyl 3-(2-ethoxy-2-oxoethoxy)-2-naphthoate (**2a**; 783 mg, >95%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.30 (t, J = 7.07 Hz, 3 H) 3.97 (s, 3H) 4.29 (q, J = 7.07 Hz, 2H) 4.81 (s, 2H) 7.14 (s, 1H) 7.37–7.45 (m, 1H) 7.49–7.56 (m, 1H) 7.72 (d, J = 8.08 Hz, 1H) 7.84 (d, J = 8.08 Hz, 1H) 8.37 (s, 1H). MS (ESI, pos.) calcd for $C_{16}H_{16}O_5$ m/z [M+H]= 288.10, found 288.09.

Compound **2a** (780 mg, 2.70 mmol) was dissolved in 2 mL THF and 6 mL of 1 N LiOH. The mixture was stirred at room temperature for 16 h. THF was removed under reduced pressure and the solution was acidified with 1 N HCl slowly. The white precipitate was filtered, washed with water, and dried to afford compound **2** (514 mg, 77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.86 (s, 2H) 7.37 (s, 1H) 7.42 (t, J = 7.45 Hz, 2H) 7.55 (t, J = 7.33 Hz, 1H) 7.83 (d, J = 8.08 Hz, 1H) 7.96 (d, J = 7.83 Hz, 1H) 8.25 (s, 1H). HRMS: calcd for $C_{13}H_{10}O_5$ + H+, 247.06010; found (ESI-FTMS, $[M+H]^{1+}$), 247.0596.

5.2.3. 3-(Carboxymethoxy)furo[2,3-*b*]pyridine-2-carboxylic acid (3). Following the literature procedure,[24] ethyl 2-chloronicotinate (1.0 g, 5.4 mmol) was converted to methyl 3-hydroxyfuro[2,3-*b*]pyridine-2-carboxylate (**3a**; 1.07 g, >95%) as a crude, off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.03 (s, 3H) 7.33 (dd, J = 7.83, 4.80 Hz, 1H) 8.13 (dd, J = 7.83, 1.77 Hz, 1H) 8.55 (dd, J = 4.80, 1.77 Hz, 1H).

A solution of **3a** (200 mg, 1 mmol), K_2CO_3 (143 mg, 1 mmol), and *tert*-butyl bromoacetate (153 mL, 1 mmol) in acetone was heated at reflux for 16 h. The cooled solution was evaporated, the residue was dissolved in ethyl acetate and water, and the organic phase was dried and evaporated. The crude produce was purified by flash chromatography using 20% ethyl acetate/hexane as eluent to give methyl 3-(2-*tert*-butoxy-2-oxoethoxy)furo[2,3-*b*]pyridine-2-carboxylate (**3b**; 116 mg, 38%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.46 (s, 9H) 3.98 (s, 3H) 4.99 (s, 2H) 7.31 (dd, J = 7.83, 4.80 Hz, 1 H) 8.19 (dd, J = 7.83, 1.77 Hz, 1H) 8.52 (dd, J = 4.80, 1.77 Hz, 1H).

A solution of **3b** (155 mg, 0.4 mmol), LiOH hydrate (63 mg, 4 equiv) in THF (1 mL), MeOH (1 mL), and water (1 mL) was stirred at room temperature for 3 h. The volatiles were evaporated, the white residue was dissolved in water (10 mL), and 10% HCl was added dropwise until the pH ~4. The resulting precipitate was filtered and dried to provide **3** (39 mg, 44%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.12 (s, 2H) 7.47 (dd, J = 7.83, 4.80 Hz, 1H) 8.31 (dd, J = 7.83, 1.52 Hz, 1H) 8.52 (dd, J = 4.80, 1.52 Hz, 1H); HRMS:

calcd for $C_{10}H_7NO_6$ + H+, 238.03462; found (ESI-FTMS, $[M+H]^{1+}$), 238.0344.

5.2.4. 3-(Carboxymethoxy)benzo[*b*]thiophene-2-carboxylic acid (4). To a solution of 3-methoxycarbonylmethoxybenzo[*b*]thiophene-2-carboxylic acid methyl ester (100 mg, 0.36 mmol) in THF (2.0 mL) was added 2.5 M LiOH in H_2O (0.3 mL). The reaction mixture was stirred vigorously for 24 h before adding 1 N HCl (1.5 mL). The solution was diluted with H_2O (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure and then triturated with diethyl ether to give **4** as a white solid (59 mg, 66%). ^{1}H NMR (400 MHz, MeOD) δ ppm 5.23 (s, 2H) 7.64–7.70 (m, 1H) 7.72–7.79 (m, 1H) 8.05 (d, J = 8.08 Hz, 1H) 8.23–8.30 (m, 1H); MS (ESI, neg.) Calcd for $C_{11}H_8O_5S$ *m*/*z* [M−H] = 251.01, found 251.09.

5.2.5. 3-(Carboxymethoxy)-6-methylthieno[3,2-*c*]pyridine-2-carboxylic acid (5). Mercaptoacetic acid methyl ester (0.19 mL, 2.14 mmole) and sodium methoxide (289 mg, 5.35 mmole) were dissolved in 40 mL DMF. The mixture was stirred at room temperature for 5 min. 2,4-Dichloro-6-methyl-nicotinic acid ethyl ester (0.5 g, 2.14 mmole) in 10 mL DMF was then added. The mixture was stirred at room temperature for 2 h. *tert*-Butyl bromoacetate (0.43 mL, 3.21 mmole) was then added and the mixture was stirred at 70 °C for 16 h. DMF was removed under reduced pressure. DCM (20 mL) was added and the organic layer was washed with water, saturated $NaHCO_3$, brine, and then dried over anhydrous Na_2SO_4. The crude product was purified by flash chromatography using ethyl acetate/hexane (0–20%) as eluent. Pure fractions were combined and evaporated to give methyl 3-(2-*tert*-butoxy-2-oxoethoxy)-4-chloro-6-methylthieno[3,2-*c*]pyridine-2-carboxylate (**5a**; 404 mg, 51% two steps) as a yellowish green solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H) 2.63 (s, 3H) 3.92 (s, 3H) 4.86 (s, 2H) 7.44 (s, 1H); MS (ESI, pos.) calcd for $C_{16}H_{18}ClNO_5S$ *m*/*z* [M+H] = 372.06, found 372.18.

Methyl 4-chloro-3-(2-ethoxy-2-oxoethoxy)-6-methyl-thieno[3,2-*c*]pyridine-2-carboxylate (**5a′**) was synthesized following the procedure in the preparation of **5a**.

A solution of **5a′** (640 mg, 1.9 mmol) in 10 mL MeOH/EtOAc (1:1) was shaken under a hydrogen atmosphere at 45 psi in the presence of 10% Pd/C (0.5 g) for 16 h. The mixture was filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography eluting with a gradient from 10% to 75% ethyl acetate in hexane. Pure fractions were combined and evaporated to give methyl 3-(2-ethoxy-2-oxoethoxy)-6-methylthieno[3,2-*c*]pyridine-2-carboxylate (**5b**; 134 mg, 23%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.27 (t, J = 7.07 Hz, 3H) 2.68 (s, 3H) 3.91 (s, 3H) 4.24 (q, J = 7.07 Hz, 2H) 5.09 (s, 2H) 7.49 (s, 1H) 9.22 (s, 1H).

Compound **5b** (92 mg, 0.3 mmol) and LiOH (56 mg, 2.34 mmol) were suspended in 2 mL THF and 3 mL water. The mixture was stirred at room temperature overnight. The reaction was acidified with 1 N HCl slowly. A white precipitate was collected, washed with water, and dried to give compound **5** (28 mg, 35%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.58 (s, 3H) 5.04 (s, 1H) 7.84 (s, 1H) 9.07 (s, 1H); HRMS: calcd for $C_{11}H_9NO_5S$ + H+, 268.02742; found (ESI-FTMS, $[M+H]^{1+}$), 268.0273.

5.2.6. 3-(Carboxymethoxy)thieno[3,2-*b*]pyridine-2-carboxylic acid (6). A solution of 3-mercaptopicolinic acid (750 mg, 3.9 mmol) and hydrochloric acid (3 drops) in methanol (100 mL) was heated at reflux for 48 h. The cooled solution was neutralized with aqueous sodium hydroxide and sodium bicarbonate, and evaporated to provide crude methyl 3-mercaptopicolinate (**6a**; 662 mg, >95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.02–4.03 (m, 3H) 7.29 (dd, J = 8.08, 4.55 Hz, 1H) 7.69 (dd, J = 8.34, 1.52 Hz, 1 H) 8.50 (dd, J = 4.29, 1.52 Hz, 1H).

A solution of **6a** (149 mg, 0.88 mmol), ethyl bromoacetate (367 mg, 2.5 equiv), and potassium carbonate (520 mg) in DMF was heated at 90 °C for 48 h. The cooled solution was acidified with aqueous hydrochloric acid, diluted with water (20 mL), and extracted with ethyl acetate (3× 30 mL). The combined organic layers were washed with saturated aqueous sodium chloride and dried with $MgSO_4$. The crude product was purified by flash chromatography (10–75% ethyl acetate/hexane) to provide ethyl 3-(2-ethoxy-2-oxoethoxy)thieno[3,2-*b*]pyridine-2-carboxylate (**6b**; 66 mg, 24%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.24 (t, J = 7.20 Hz, 3H) 1.41 (t, J = 7.07 Hz, 3H) 4.23 (q, J = 7.07 Hz, 2H) 4.41 (q, J = 7.07 Hz, 2H) 5.47 (s, 2H) 8.08 (dd, J = 8.34, 1.52 Hz, 1H) 8.66 (dd, J = 4.55, 1.52 Hz, 1H).

A solution of **6b** (55 mg, 0.18 mmol) and LiOH hydrate (37 mg, 5 equiv) in THF (2 mL) and water (2 mL) was stirred at room temperature for 18 h. The solution was evaporated, acidified to pH 4, and cooled to 0 °C. The resulting precipitate was collected by filtration and dried under vacuum to provide **7** (4 mg, 9%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.29 (s, 2H) 7.34 (dd, J = 8.21, 4.42 Hz, 1H) 7.50 (dd, J = 8.34, 4.55 Hz, 1H) 8.46 (dd, J = 8.34, 1.52 Hz, 1H) 8.69 (dd, J = 4.55, 1.52 Hz, 1H).

5.2.7. 3-(Carboxymethoxy)thieno[3,2-*b*]thiophene-2-carboxylic acid (7). A solution of methyl 3-chlorothiophene-2-carboxylate (2.75 g, 15.6 mmol) methyl thioglycolate (1.42 mL, 15.6 mmol) and potassium carbonate (4.74 g, 31.2 mmol) in DMF (60 mL) was heated at 60 °C for 18 h. The cooled solution was diluted with water (100 mL) and extracted with ethyl acetate (3× 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, evaporated, and purified by flash chromatography (2–35% ethyl acetate/hexane) to provide methyl 3-(2-methoxy-2-oxoethylthio)thiophene-2-carboxylate (**7a**; 81 mg, 5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.75 (s, 3H) 3.76 (s, 2H) 3.89 (s, 3H) 7.09 (d, J = 5.31 Hz, 1H) 7.26 (s, 1H) 7.51 (d, J = 5.31 Hz, 1H).

A solution of **7a** (240 mg, 0.97 mmol) and sodium *tert*-butoxide (230 mg, 2.5 equiv) in DMF (7 mL) was heated

at 60 °C for 18 h. The cooled solution was acidified with aqueous hydrochloric acid and extracted with ethyl acetate (3× 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to provide methyl 3-hydroxythieno[3,2-*b*]thiophene-2-carboxylate (**7b**; 163 mg, 76%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.93 (s, 3 H) 7.21 (d, J = 5.31 Hz, 1H) 7.63 (d, J = 5.31 Hz, 1 H).

A solution of **7b** (140 mg, 0.85 mmol) ethyl bromoacetate (100 μL, 0.94 mmol) and sodium *tert*-butoxide (75 mg, 0.78 mmol) in DMF (3 mL) was heated at 40 °C for 2 h. The cooled solution was acidified with aqueous hydrochloric acid and extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to provide methyl 3-(2-ethoxy-2-oxoethoxy)thieno[3,2-*b*]thiophene-2-carboxylate (**7c**; 195 mg, 64%) as a pale-brown liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.28 (t, J = 7.07 Hz, 3H) 3.89 (s, 3H) 4.26 (q, J = 7.07 Hz, 2H) 5.03 (s, 2H) 7.19 (d, J = 5.31 Hz, 1H) 7.58 (d, J = 5.31 Hz, 1H).

A solution of **7c** (71 mg, 0.24 mmol) and LiOH hydrate (37 mg, 0.88 mmol) in THF (2 mL) and water (2 mL) was heated at 40 °C for 4 h. The cooled solution was evaporated, and the resulting aqueous mixture was acidified with hydrochloric acid. The resulting yellow precipitate, compound **7** (60 mg, 98%), was collected by filtration. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.54 (s, 2 H) 7.45 (d, J = 5.31 Hz, 1H) 7.85 (d, J = 5.31 Hz, 1 H); HRMS: calcd for C9H6O5S2 + H+, 258.97294; found (ESI-FTMS, [M+H]1+), 258.9726.

5.2.8. 3-Carboxymethoxy-6-chloro-benzo[*b*]-thiophene-2-carboxylic acid (8). A solution of 6-chloro-3-hydroxy-benzo[*b*]thiophene-2-carboxylic acid methyl ester (5.28 g, 21.8 mmol), K_2CO_3 (4.51 g, 32.7 mmol), and *tert*-butyl bromoacetate (4.2 mL 28.3 mmol) in 160 mL DMF was stirred at 50 °C for 18 h. Water was added and the mixture was extracted with ether (3× 75 mL). The organic layers were combined, washed with brine, dried over anhydrous $MgSO_4$, and filtered. Solvent was removed under reduced pressure to give 3-*tert*-butoxycarbonylmethoxy-6-chloro-benzo[*b*]thiophene-2-carboxylic acid methyl ester (**8a**; 7.70 g, 99%) as slightly pink solids. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.46 (s, 9H) 3.91 (s, 3H) 4.92 (s, 2H) 7.38 (dd, J = 8.59, 2.02 Hz, 1H) 7.71 (d, J = 2.02 Hz, 1H) 8.03 (d, J = 8.59 Hz, 1H).

Compound **8a** (100 mg, 0.28 mmol) was hydrolyzed following the procedure in the preparation of **1** to give compound **8** (72 mg, 90%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.00 (s, 2H) 7.52 (d, J = 8.59 Hz, 1H) 7.97 (d, J = 8.59 Hz, 1H) 8.14 (s, 1H).

5.2.9. 3-(1-Carboxy-ethoxy)-6-chloro-benzo[*b*]-thiophene-2-carboxylic acid (9). 6-Chloro-3-hydroxy-benzo[*b*]thiophene-2-carboxylic acid methyl ester (200 mg, 0.82 mmol) was dissolved in 10 mL DMF, followed by addition of K_2CO_3 (342 mg, 2.47 mmol) and 2-bromo-propionic acid methyl ester (110 μL, 0.99 mmol). The mixture was stirred at 70 °C for 16 h. DMF was evaporated under reduced pressure followed by addition of 15 mL DCM. The organic layer was washed with water, brine, and dried over anhydrous Na_2SO_4. The crude product was purified by flash chromatography eluting with a gradient of ethyl acetate in hexane. Pure fractions were combined and evaporation of solvents gave 6-chloro-3-(1-methoxycarbonyl-ethoxy)-benzo[*b*]thiophene-2-carboxylic acid methyl ester (**9a**; 254 mg, 94%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.71 (d, J = 6.82 Hz, 3H) 3.70 (s, 3H) 3.90 (s, 3H) 5.30 (q, J = 6.82 Hz, 1H) 7.37 (dd, J = 8.59, 1.77 Hz, 1H) 7.70 (d, J = 1.77 Hz, 1H) 7.98 (d, J = 8.59 Hz, 1H).

Compound **9a** (250 mg, 0.76 mmol) was hydrolyzed following the procedure in the preparation of **1** to give compound **9** (211 mg, 92%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.56 (d, J = 6.82 Hz, 3H) 5.26 (q, J = 6.82 Hz, 1H) 7.50 (dd, J = 8.59, 1.77 Hz, 1H) 7.95 (d, J = 8.59 Hz, 1H) 8.13 (d, J = 1.77 Hz, 1H). HRMS: calcd for $C_{12}H_9ClO_5S$ + H+, 300.99320; found (ESI-FTMS, $[M+H]^{1+}$), 300.9934.

5.2.10. 3-(Carboxy-fluoro-methoxy)-6-chloro-benzo[*b*]thiophene-2-carboxylic acid (10). 6-Chloro-3-hydroxy-benzo[*b*]thiophene-2-carboxylic acid methyl ester (200 mg, 0.824 mmol) was dissolved in 10 mL DMF, followed by addition of K_2CO_3 (342 mg, 2.47 mmol) and bromo-fluoro-acetic acid ethyl ester (117 μL, 0.99 mmol). The mixture was stirred at 70 °C for 16 h. DMF was evaporated under reduced pressure, followed by addition of 15 mL DCM. The organic layer was washed with water, brine, and dried over anhydrous Na_2SO_4. The crude product was purified by flash chromatography eluting with a gradient of ethyl acetate in hexane. Pure fractions were combined and evaporation of solvents gave 6-chloro-3-(ethoxycarbonyl-fluoro-methoxy)-benzo[*b*]thiophene-2-carboxylic acid methyl ester (**10a**; 161 mg, 56%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.43 (t, J = 7.07 Hz, 3H) 3.93 (s, 3H) 4.44 (q, J = 7.07 Hz, 2H) 6.17 (d, J = 59.37 Hz, 1H) 7.42 (dd, J = 8.59, 1.77 Hz, 1H) 7.76 (d, J = 1.77 Hz, 1H) 7.91 (d, J = 8.59 Hz, 1H).

Compound **10a** (81 mg, 0.23 mmol) was hydrolyzed following the procedure in the preparation of **1** to give compound **10** (67 mg, 95%) as a pink solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 6.23 (d, J = 57.35 Hz, 1H) 7.55 (d, J = 8.34 Hz, 2 H) 7.81 (d, J = 8.34 Hz, 2H) 8.20 (s, 1H). HRMS: calcd for $C_{11}H_6ClFO_5S$ + H+, 304.96813; found (ESI-FTMS, $[M+H]^{1+}$), 304.9684.

5.2.11. 6-Bromo-3-carboxymethoxy-benzo[*b*]-thiophene-2-carboxylic acid (11). LiOH monohydrate (2.0 g, 48 mmol) was added to a solution of 4-bromo-2-fluoro-benzoic acid methyl ester (5.5 g, 24 mmol) and methyl thioglycolate (2.1 mL, 24 mmol) in 30 mL DMF at 0 °C. The mixture was stirred at 0 °C for 30 min, warmed to room temperature, added to water, and acidified with 1 N HCl. The precipitate was filtered, washed with water, and dried to give 6-bromo-3-hydroxy-benzo[*b*]thiophene-2-carboxylic acid methyl ester (**11a**; 4.89 g, 72%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.81 (s, 3H) 7.55 (d, J = 8.34, 1.26 Hz, 1H) 7.82 (d, J = 8.34 Hz, 1H) 8.19 (s, 1H).

Ethyl bromoacetate (3.0 mL, 27 mmol) was added to a mixture of **11a** (5.1 g, 18 mmol) and sodium *tert*-butoxide (1.9 g, 20 mmol) in 75 mL DMF. The mixture was stirred at 80 °C overnight. Water was then added and a precipitate emerged. The solid was filtered, washed with water, and recrystallized in MeOH to give 6-bromo-3-ethoxycarbonylmethoxy-benzo[*b*]thiophene-2-carboxylic acid methyl ester (**11b**; 4.5 g, 67%) as a slightly pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.27 (t, J = 7.07 Hz, 3H) 3.91 (s, 3H) 4.24 (q, J = 7.07 Hz, 2 H) 5.02 (s, 2H) 7.53 (dd, J = 8.59, 1.77 Hz, 1H) 7.89 (dd, J = 1.77, 0.51 Hz, 1H) 7.96 (dd, J = 8.59, 0.51 Hz, 1 H).

Compound **11b** (100 mg, 0.27 mmol) was hydrolyzed following the procedure in the preparation of **1** to give compound **11** (73 mg, 82%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.99 (s, 2H) 7.64 (dd, J = 8.59, 1.77 Hz, 1H) 7.90 (dd, J = 8.59, 0.51 Hz, 1H) 8.29 (dd, J = 1.77, 0.51 Hz, 1H). HRMS: calcd for $C_{11}H_7BrO_5S$ + H+, 330.92703; found (ESI-FTMS, $[M+H]^{1+}$), 330.9272.

5.2.12. 3-Carboxymethoxy-6-phenylbenzo[*b*]-thiophene-2-carboxylic acid (12). A solution of 6-chloro-3-hydroxybenzo[*b*]thiophene-2-carboxylic acid methyl ester (5.28 g, 21.8 mmol), K_2CO_3 (4.51 g, 32.7 mmol), and *tert*-butyl bromoacetate (4.2 mL, 28.3 mmol) in 160 mL DMF was stirred at 50 °C for 18 h. Water was added and the mixture was extracted with ether (3× 75 mL). The organic layers were combined, washed with brine, dried over anhydrous $MgSO_4$, and filtered. Solvent was removed under reduced pressure to give 3-*tert*-butoxycarbonylmethoxy-6-chloro-benzo[*b*]thiophene-2-carboxylic acid methyl ester (**12a**; 7.70, 99%) as slightly pink solids. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.46 (s, 9H) 3.91 (s, 3H) 4.92 (s, 2H) 7.38 (dd, J = 8.59, 2.02 Hz, 1H) 7.71 (d, J = 2.02 Hz, 1H) 8.03 (d, J = 8.59 Hz, 1H).

Compound **12a** (90 mg, 0.25 mmol), $Pd_2(dba)_3$ (4.4 mg, 0.0048 mmol), $HP(t\text{-}Bu)_3BF_4$ (2.9 mg, 0.01 mmol), KF (43 mg, 0.75 mmol), and phenyl boronic acid (34 mg, 0.28 mmol) in 2 mL THF under nitrogen were stirred at 60 °C for 5 h. The crude mixture was absorbed onto silica and purified by flash chromatography to give 3-*tert*-butoxycarbonylmethoxy-6-phenylbenzo[*b*]-thiophene-2-carboxylic acid methyl ester (**12b**: 20 mg, 20%) as a white solid. Compound **12b** (20 mg, 0.05 mmol) was then hydrolyzed following the procedure in the preparation of **1** to give compound **12** (6 mg, 38%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.00 (s, 2H) 7.42 (m, 1H) 7.51 (t, J = 7.58 Hz, 2H) 7.79 (m, 3H) 8.05 (d, J = 8.34 Hz, 1H) 8.26 (d, J = 0.76 Hz, 1H). HRMS: calcd for $C_{17}H_{12}O_5S$ + H+, 329.04782; found (ESI-FTMS, $[M+H]^{1+}$), 329.048.

5.2.13. 3-Carboxymethoxy-6-(4-hydroxyphenyl)-benzo[*b*]thiophene-2-carboxylic acid (13). Following the procedure in the preparation of **12b**, **12a** (102 mg, 0.29 mmol), 4-hydroxyphenylboronic acid (51 mg, 0.37 mmol), $Pd(OAc)_2$ (8 mg, 0.036 mmol), 2-(dicyclohexylphosphino)-biphenyl (24 mg, 0.068 mmol), and KF (50 mg, 0.86 mmol) were used and the reaction mixture was stirred at 60 °C until TLC showed the absence of **12a**. The crude product was purified by flash chromatography to give 3-*tert*-butoxycarbonylmethoxy-6-(4-hydroxy-phenyl)-benzo[*b*]thiophene-2-carboxylic acid methyl ester (**13a**; 55 mg, 47%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.48 (s, 9H) 3.92 (s, 3H) 4.89 (s, 1H) 4.93 (s, 2H) 6.93 (d, J = 8.84 Hz, 2H) 7.54 (d, J = 8.84 Hz, 2H) 7.60 (dd, J = 8.34, 1.52 Hz, 1H) 7.85 (d, J = 1.52 Hz, 1H) 8.12 (d, J = 8.34 Hz, 1H).

Compound **13a** (50 mg, 0.12 mmol) was hydrolyzed following the procedure in the preparation of **1** to give compound **13** (29 mg, 69%) as a light-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.00 (s, 2H) 6.88 (d, J = 8.84 Hz, 2H) 7.61 (d, J = 8.84 Hz, 2H) 7.71 (d, J = 8.59, 1H) 7.99 (d, J = 8.59 Hz, 1H) 8.14 (s, 1H) 9.67 (s, 1H). HRMS: calcd for $C_{17}H_{12}O_6S$ + H+, 345.04274; found (ESI-FTMS, $[M+H]^{1+}$), 345.0428.

5.2.14. 3-Carboxymethoxy-6-thiophen-2-yl-benzo[*b*]-thiophene-2-carboxylic acid (14). Following the procedure in the preparation of **12b**, **12a** (90 mg, 0.25 mmol, 1 equiv), 2-thiophene boronic acid (65 mg, 0.5 mmol), $Pd[P(t\text{-}Bu)_3]_2$ (20 mg, 0.039 mmol), and KF (50 mg, 0.86 mmol) were used and the reaction mixture was stirred at 60 °C for 48 h. The crude product was purified by flash chromatography to yield 3-*tert*-butoxycarbonylmethoxy-6-thiophen-2-yl-benzo[*b*]thiophene-2-carboxylic acid methyl ester (**14a**; 86 mg, 62%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H) 3.92 (s, 3H) 4.93 (s, 2H) 7.12 (dd, J = 5.05, 3.54 Hz, 1H) 7.35 (dd, J = 5.05, 1.14 Hz, 1H) 7.42 (dd, J = 3.54, 1.14 Hz, 1H) 7.68 (dd, J = 8.34, 1.52 Hz, 1H) 7.94 (dd, J = 1.52, 0.63 Hz, 1H) 8.09 (dd, J = 8.34, 0.63 Hz, 1H).

Compound **14a** (71 mg, 0.18 mmol) was hydrolyzed following the procedure in the preparation of **1** to give compound **14** (4 mg, 7%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.00 (s, 2H) 7.19 (dd, J = 5.05, 3.54 Hz, 1H) 7.64 (dd, J = 5.05, 1.14 Hz, 1H) 7.68 (dd, J = 3.54, 1.14 Hz, 1 H) 7.78 (dd, J = 8.34, 1.52 Hz, 1H) 7.99 (d, J = 8.34 Hz, 1H) 8.27 (d, J = 1.52 Hz, 1H).

5.2.15. 3-Carboxymethoxy-6-(5-methyl-1-phenyl-1*H*-pyrazol-3-ylcarbamoyl)-benzo[*b*]thiophene-2-carboxylic acid (15). A solution of 4-(methoxycarbonyl)-3-nitrobenzoic acid (5.0 g, 22 mmol), isobutylene (20 mL), and concentrated sulfuric acid (1 mL) in dioxane (25 mL) was sealed in a Parr bottle and shaken at room temperature overnight. A needle was inserted through the silicone stopper to release excessive pressure. The vessel was carefully removed from the shaker apparatus and the reaction solution was stirred open to the atmosphere for 1 h. The solution was diluted with ethyl acetate (300 mL) and water (50 mL), neutralized with aqueous sodium hydroxide, washed with water and brine, and dried over anhydrous $MgSO_4$. Evaporation of solvent yielded *tert*-butyl 4-(methoxycarbonyl)-3-nitrobenzoate (**15a**; 3.38 g, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.62 (s, 9 H) 3.95 (s, 3H) 7.79 (d, J = 7.83 Hz, 1H) 8.27 (dd, J = 7.83, 1.52 Hz, 1H) 8.48 (d, J = 1.52 Hz, 1H).

A solution of **15a** (3.3 g, 12 mmol) and methyl thioglycolate (1.6 mL, 8.0 mmol) in 15 mL DMF was cooled to 0 °C, and LiOH monohydrate (0.99 g, 24 mmol) was added over a 15 min period. The reaction mixture was stirred at 0 °C for an additional 30 min, allowed to warm to room temperature, and stirred an additional 2 h. The solution was acidified, diluted with ethyl acetate (300 mL), and washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to provide 6-*tert*-butyl 2-methyl 3-hydroxybenzo[*b*]thiophene-2,6-dicarboxylate (**15 b**; 3.10 g, 84%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.63 (s, 9H) 3.97 (s, 3H) 7.96 (d, J = 8.4 Hz, 1H) 8.00 (d, J = 8.4 Hz, 1H) 8.39 (s, 1 H).

Compound **15b** (3.1 g, 10 mmol) was alkylated with methyl bromoacetate following the procedure in the preparation of **12a** to give 6-*tert*-butyl 2-methyl 3-(2-methoxy-2-oxoethoxy)benzo[*b*]thiophene-2,6-dicarboxylate (**15c**; 3.17 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.61 (s, 9H) 3.77 (s, 3H) 3.91 (s, 3H) 5.02 (s, 2H) 7.99 (d, J = 8.80 Hz, 1H) 8.08 (d, J = 8.80 Hz, 1H) 8.37 (s, 1H).

A solution of **15c** (2.80 g, 7.4 mmol) and trifluoroacetic acid (6 mL) in DCM (40 mL) was stirred at room temperature for 4.5 h. Solvents were removed under reduced pressure to give 3-(2-methoxy-2-oxoethoxy)-2-(methoxycarbonyl)benzo[*b*]thiophene-6-carboxylic acid (**15d**; 2.60 g, >95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.69 (s, 3H) 3.87 (s, 3H) 5.10 (s, 2H) 8.00 (d, J = 8.34 Hz, 1H) 8.09 (d, J = 8.34 Hz, 1H) 8.62 (s, 1H).

A solution of **15d** (0.97 g, 3 mmol) and thionyl chloride (10 mL) in toluene (50 mL) was heated at reflux overnight. The cooled solution was concentrated in vacuo, and the residual thionyl chloride was removed by azeotroping with added toluene. The crude acid chloride, methyl 6-(chlorocarbonyl)-3-(2-methoxy-2-oxoethoxy)benzo[*b*]-thiophene-2-carboxylate (**15e**), was used without additional purification. A solution of **15e** (75 mg, 0.22 mmol), 3-amino-5-methyl-1-phenylpyrazole (57 mg, 0.33 mmol), and pyridine (53 μL, 0.66 mmol) in DCM (3 mL) was stirred at room temperature for 4 h. The reaction was quenched with aqueous sodium bicarbonate, diluted with ethyl acetate (50 mL) and washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to provide a crude yellow solid. The crude product was recrystallized from ethanol to give 3-methoxycarbonylmethoxy-6-(5-methyl-1-phenyl-1*H*-pyrazol-3-ylcarbamoyl)-benzo[*b*]thiophene-2-carboxylic acid methyl ester (**15f**; 53 mg, 50%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.33 (s, 3H) 3.69 (s, 3H) 3.87 (s, 3H) 5.11 (s, 2H) 6.31 (s, 1H) 7.31 (m, 1H) 7.45 (m, 2H) 7.54 (m, 2H) 7.92 (d, J = 8.59 Hz, 1H) 8.10 (d, J = 8.59 Hz, 1H) 8.49 (s, 1H) 10.57 (s, 1H).

Compound **15f** (53 mg, 0.11 mmol) was hydrolyzed following the procedure in the preparation of **1** to give compound **15** (39 mg, 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.26 (s, 3 H) 5.02 (s, 2H) 6.31 (s, 1H) 7.31 (m, 1H) 7.45 (m, 5H) 7.54 (m, 2H) 7.89 (m, 1H) 8.07 (d, J = 8.59 Hz, 1H) 8.45 (s, 1H) 10.54 (s, 1H). HRMS (ESI) calcd for $C_{22}H_{17}N_3O_6S$ 452.09109; found 452.08967.

5.2.16. 3-Carboxymethoxy-7-chloro-benzo[*b*]-thiophene-2-carboxylic acid (16). Following the procedure in the preparation of **15b**, 3-chloro-2-nitrobenzoic acid methyl ester (1.98 g, 9.2 mmol) was converted to 7-chloro-3-hydroxybenzo[*b*]thiophene-2-carboxylic acid methyl ester (**16a**; 1.12 g, 50%) after recrystallization from ethanol. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.98 (s, 3H) 7.38 (t, J = 7.83 Hz, 1H) 7.49–7.54 (m, 1H) 7.87 (dd, J = 8.08, 1.01 Hz, 1H) 10.09 (s, 1H).

Following the procedure in the preparation of **12a**, **16a** (1.12 g, 4.62 mmol) was converted to 7-chloro-3-methoxycarbonylmethoxy-benzo[*b*]thiophene-2-carboxylic acid methyl ester (**16b**; 1.34 g, 92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.69 (s, 3H) 3.88 (s, 3H) 5.12 (s, 2H) 7.57 (t, J = 7.83 Hz, 1H) 7.75 (dd, J = 7.71, 0.88 Hz, 1H) 8.01 (dd, J = 8.08, 0.88 Hz, 1H).

Compound **16 b** (100 mg, 0.32 mmol) was hydrolyzed following the procedure in the preparation of **1** to give compound **16** (65 mg, 71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.02 (s, 2 H) 7.53 (t, J = 7.83 Hz, 1H) 7.70 (dd, J = 6.82, 1.01 Hz, 1H) 7.98 (dd, J = 8.08, 1.01 Hz, 1H). HRMS (ESI) calcd for $C_{11}H_6ClO_5S$ 284.96299; found 284.96231.

5.2.17. 3-Carboxymethoxy-7-methyl-benzo[*b*]-thiophene-2-carboxylic acid (17). 3-Methyl-2-nitro-benzoic acid methyl ester (3.2 g, 17 mmol) was converted to 3-hydroxy-7-methyl-benzo[*b*]thiophene-2-carboxylic acid methyl ester, which was subsequently converted to 3-methoxycarbonylmethoxy-7-methylbenzo[*b*]thiophene-2-carboxylic acid methyl ester (**17a**; 1.56 g, 32% 2 steps), following the procedure in the preparation of **15b** and **12a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.51 (s, 3H) 3.80 (s, 3 H) 3.92 (s, 3H) 5.02 (s, 2H) 7.30 (d, J = 7.07 Hz, 1H) 7.28–7.32 (m, 1H) 7.91 (d, J = 8.08 Hz, 1H).

Compound **17a** (100 mg, 0.34 mmol) was hydrolyzed following the procedure in the preparation of **1** to give compound **17** (81 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.45 (s, 3H) 4.68 (s, 2H) 7.30 (d, J = 7.07 Hz, 1H) 7.33–7.39 (m, 1H) 7.72 (d, J = 7.83 Hz, 1H). HRMS: calcd for $C_{12}H_{10}O_5S$ + H+, 267.03217; found (ESI-FTMS, $[M+H]^{1+}$), 267.0322.

5.2.18. 3-Carboxymethoxy-naphtho[1,2-*b*]thiophene-2-carboxylic acid (18). To an oven-dried 50 mL round-bottomed flask under a nitrogen atmosphere were added 1-bromo-naphthalene-2-carboxylic acid ethyl ester (0.244 g, 0.88 mmol), methyl thioglycolate (0.08 mL, 0.88 mmol), 20 mL DMF, and then sodium hydride (0.075 g of a 60% suspension in mineral oil, 1.84 mmol). The resulting mixture was allowed to stir at room temperature for 16 h. To the solution, 25 mL of 1.2 N HCl was added, at which point a precipitate formed. The solid was filtered and dried to give 3-hydroxy-naphtho[1,2-*b*]thiophene-2-carboxylic acid methyl ester (**18a**; 0.134 g, 60%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.99 (s, 3H) 7.61 (m, 2H) 7.75 (d, J = 8.59 Hz, 1H) 7.87 (d, J = 8.84 Hz, 1H) 7.94 (dd, J = 6.06, 3.54 Hz, 1H) 8.10 (dd, J = 6.06, 3.28 Hz, 1 H) 10.12 (s, 1H).

To an oven-dried 50 mL round-bottomed flask under a nitrogen atmosphere were added **18a** (0.134 g, 0.52 mmol), ethyl bromoacetate (0.06 mL, 0.52 mmol), potassium carbonate (0.072 g, 052 mmol), and 20 mL DMF. The resulting mixture was allowed to stir for 16 h at room temperature. To the mixture, 30 mL of water was added, at which point a precipitate formed. The solid was filtered and dried to give 3-ethoxycarbonylmethoxy-naphtho[1,2-*b*]thiophene-2-carboxylic acid methyl ester (**18b**; 0.116 g, 65%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.28 (t, J = 7.20 Hz, 3H) 3.95 (s, 3H) 4.26 (q, J = 7.16 Hz, 2H) 5.07 (s, 2H) 7.58 (m, 2H) 7.76 (m, 1H) 7.93 (m, 1H) 8.02 (m, 1H) 8.11 (m, 1H).

To a 25 mL round-bottomed flask, **18b** (0.116 g, 0.34 mmol) and 5 mL THF were added, followed by addition of a solution of LiOH hydrate (0.057 g, 1.35 mmol) in 3 mL water. This mixture was allowed to stir at room temperature for 16 h. At this time 15 mL of 1.2 N HCl was added, and a precipitate formed. The solid was filtered and dried to give **18** (0.096 g, 93%) as a tan solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.06 (s, 2H) 7.69 (m, 2H) 7.91 (d, J = 8.84 Hz, 1 H) 7.95 (m, 1H) 8.08 (m, 1H) 8.19 (t, J = 4.0 Hz, 1H); HRMS: calcd for $C_{15}H_{10}O_5S$ + H+, 303.03217; found (ESI-FTMS, $[M+H]^{1+}$), 303.0325.

5.2.19. 3-Carboxymethoxy-thieno[3,2-*c*]quinoline-2-carboxylic acid (19). Following the procedure in the preparation of **18a**, except that ethyl 4-chloroquinoline-3-carboxylate was used as starting material, 3-hydroxy-thieno[3,2-*c*]quinoline-2-carboxylic acid methyl ester (**19a**) was obtained as a tan solid (84 mg, 38%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.89 (s, 3H) 7.75 (m, 1H) 7.87 (m, 1H) 8.17 (d, J = 7.83 Hz, 1H) 8.24 (d, J = 7.33 Hz, 1H) 9.29 (s, 1H) 11.18 (s, 1H).

Following the procedure in the preparation of **18b**, **19a** (84 mg, 0.32 mmol) was alkylated with ethyl bromoacetate to give 3-ethoxycarbonylmethoxy-thieno[3,2-*c*]quinoline-2-carboxylic acid methyl ester (**19b**) as a white solid (51 mg, 46%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.18 (t, J = 7.07 Hz, 3H) 3.91 (s, 3 H) 4.15 (q, J = 7.07 Hz, 2H) 5.22 (s, 2H) 7.77 (m, 1H) 7.90 (m, 1H) 8.20 (d, J = 7.83 Hz, 1H) 8.30 (d, J = 8.08 Hz, 1H) 9.34 (s, 1H).

Compound **19b** (51 mg, 0.15 mmol) was hydrolyzed according to the procedure in the preparation of **18** to give compound **19** as a white solid (22 mg, 48%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.15 (s, 2H) 7.83 (m, 2H) 8.23 (m, 2H) 9.32 (s, 1H).

5.2.20. 3-(Carboxymethoxy)thieno[3,2-*b*][1]benzo-thiophene-2-carboxylic acid (20). Sodium *tert*-butoxide (360 mg, 3.7 mmol) was added to a solution of methyl thioglycolate (0.110 mL, 1.23 mmol) in DMF (10 mL). 2-Chloro-benzo[*b*]thiophene-3-carboxylic acid methyl ester (235 mg, 1.12 mmol) was then added and the mixture became orange. When no starting material was observed by TLC, water was added followed by extraction with ethyl acetate. Purification by flash chromatography yielded methyl 3-(hydroxy)thieno[3,2-*b*][1]benzo-thiophene-2-carboxylate (**20a**; 162 mg, 61%) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.96 (s, 3H) 7.51–7.40 (dd, J = 6.06, 3.28 Hz, 2H) 7.89–7.80 (m, 2H) 10.06 (s, 1H).

tert-Butyl bromoacetate (0.082 mL, 0.56 mmol) was added to a mixture of sodium *tert*-butoxide (50 mg, 0.67 mmol) and **20a** (113 mg, 0.43 mmol) in DMF (10 mL). The resulting mixture was stirred at 60 °C for 16 h. Addition of water followed by extraction with ethyl acetate and washing with water gave the crude product. Purification by flash chromatography gave methyl 3-(*tert*-butoxycarbonylmethoxy)thieno[3,2-*b*][1]benzo-thiophene-2-carboxylate (**20b**; 140 mg, 86%) of as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H) 3.91 (s, 3H) 4.97 (s, 2H) 7.47–7.41 (m, 2H) 7.90–7.82 (m, 2H).

Compound **20b** (76 mg, 0.2 mmol) was hydrolyzed following the procedure in the preparation of **1** to give compound **19** (57 mg, 92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.69 (d, J = 1.01 Hz, 2H) 7.55–7.46 (m, 2H) 8.08–8.02 (m, 1H) 8.13–8.09(m, 1H). HRMS (ESI−, *m*/*z*) calcd for $[M-H]^{1-}$, 306.97403, found, 306.97322.

5.2.21. 3-(Carboxymethoxy)-5-chlorothieno[3,2-*b*][1]benzo-thiophene-2-carboxylic acid (21). Trifluoromethanesulfonyl anhydride (0.49 mL, 2.9 mmol) was added dropwise to a 0 °C solution of 7-chloro-3-hydroxy-benzo[*b*]thiophene-2-carboxylic acid methyl ester (355 mg, 1.46 mmol) in Et_3N (8 mL) and DCM (5 mL). After reaching room temperature, the mixture was adsorbed onto silica gel and purified by flash chromatography to yield 7-chloro-3-trifluoromethane-sulfonyloxy-benzo[*b*]thiophene-2-carboxylic acid methyl ester (**21a**; 110 mg, 20%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.01 (s, 3 H), 7.50 (t, J = 8.0 Hz, 1H), 7.56–7.65 (m, 1H), 7.77 (d, J = 8.1 Hz, 1H).

Compound **21a** (104 mg, 0.28 mmol) in DMF (2 mL) was added to a mixture of sodium *tert*-butoxide (54 mg, 0.56 mmol) and methyl thioglycolate dissolved in DMF (1 mL). After 16 h at room temperature, water was added, and the mixture was neutralized with 1 N HCl. It was then extracted with ethyl acetate, dried over $MgSO_4$, filtered, and evaporated. The resulting solid was then treated with ethyl bromoacetate (120 μl, 1.1 mmol) in the presence of K_2CO_3 (90 mg, 0.65 mmol) in DMF (2 mL) at 60 °C. After 24 h, water was added and the mixture was extracted with ethyl acetate. Removal of solvent and purification by reverse-phase HPLC yielded methyl 3-(ethoxycarbonylmethoxy)-5-chlorothieno[3,2-*b*][1]benzothiophene-2-carboxylate (**22b**; 19 mg, 16% 2 steps). Subsequent hydrolysis following the procedure in the preparation of **1** yielded compound **21** (11 mg, 65%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.67 (s, 2H) 7.56 (t, J = 7.83 Hz, 1H) 7.63 (dd, J = 7.83, 1.01 Hz, 1H) 8.07 (dd, J = 7.83, 1.01 Hz, 1H). HRMS: calcd for $C_{13}H_7ClO_5S_2-H+$, 340.93507; found (ESI−, $[M-H]^{1-}$), 340.9345.

5.2.22. 3-(Carboxymethoxy)-6-chlorothieno[3,2-*b*][1]benzothiophene-2-carboxylic acid (22). 6-Chloro-2-hydroxy-benzo[*b*]thiophene-3-carboxylic acid methyl ester

(2.46 g, 10.1 mmol) was cooled to 0 °C in a solution of DCM (25 mL) and Et_3N (25 mL). Trifluoromethanesulfonyl anhydride (2.56 mL, 15.2 mmol) was added dropwise and the mixture was stirred at room temperature for 1 h. The resulting solution was washed with water, saturated $NaHCO_3$, and dried over $MgSO_4$. The crude material was purified by column chromatography to give methyl 6-chloro-2-trifluoromethane-sulfonyloxybenzo[*b*]thiophene-3-carboxylate (**22a**; 2.61 g, 68%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.00 (s, 3H), 7.50 (dd, J = 8.8, 1.8 Hz, 1H), 7.76 (d, J = 8.8 Hz, 1H), 7.85 (d, J = 1.5 Hz, 1H)

Methyl thioglycolate (0.169 mL, 1.9 mmol) was added to a solution of **22a** (0.644 g, 1.72 mmol) in Et_3N (10 mL) and DMF (5 mL), and the mixture was stirred at room temperature for 16 h. Addition of water followed by extraction with ethyl acetate, drying over $MgSO_4$, and removal of solvent yielded 0.564 g of crude product. NaH 60% in oil (65 mg) was added to 449 mg of the crude product in DMF (8 mL) and stirred for 30 min. Water was added and the mixture was acidified with 1 N HCl. Extraction with ethyl acetate, drying over $MgSO_4$, and removal of solvent gave methyl 3-(hydroxy)-6-chlorothieno[3,2-*b*][1]benzothiophene-2-carboxylate (**22b**; 0.173 g, 43%) as a light orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.96 (s, 3H) 7.42 (dd, J = 8.59, 1.77 Hz, 1H) 7.79 (d, J = 8.59 Hz, 1H) 7.88 (d, J = 1.77 Hz, 1H) 10.02 (s, 1H). HRMS (ESI−, *m*/*z*) Calcd for $[M-H]^{1-}$, 296.94523, found, 296.4479.

tert-Butyl bromoacetate (0.10 mL, 0.65 mmol) was added to a mixture of sodium *tert*-butoxide (60 mg, 0.63 mmol) and **22b** (150 mg, 0.5 mmol) in DMF (6 mL), and the mixture was stirred at 60 °C for 2.5 h and then at room temperature for 3 days. Water was added and the mixture was extracted with ethyl acetate. After removal of solvent, purification by flash chromatography gave methyl 3-(*tert*-butoxycarbonylmethoxy)-6-chlorothieno-[3,2-*b*][1]benzothiophene-2-carboxylate (**22c**; 140 mg, 68%) of as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9 H), 4.95 (s, 2H), 7.41 (dd, J = 8.6, 2.0 Hz, 1H), 7.77 (d, J = 8.6 Hz, 1H), 7.83 (d, J = 2.0 Hz, 1H).

Compound **22c** (48 mg, 0.12 mmol) was hydrolyzed following the procedure in the preparation of **1** to yield compound **22** (26 mg, 65%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.06 (s, 2H) 7.55 (dd, J = 8.59, 2.02 Hz, 1H) 8.12 (d, J = 8.59 Hz, 1H) 8.32 (d, J = 2.02 Hz, 1H). HRMS: calcd for $C_{13}H_7ClO_5S_2-H+$, 340.93507; found (ESI−, $[M-H]^{1-}$), 340.93465.

5.2.23. 3-(Carboxymethoxy)-5-[(cyclohexylmethyl)-amino]thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid (23). To a solution of 2-nitro-isophthalic acid (5.0 g, 23.7 mmol) in DMF (80 mL) were added allyl bromide (10.3 mL, 118.5 mmol) and potassium carbonate (30 g, 237 mmol). The mixture was heated to 50 °C and allowed to stir for 3 h, after which the reaction was judged complete by TLC. The reaction was quenched with water and the product was extracted into ethyl acetate (3× 50 mL). The combined organic layers were washed with water, dried over $MgSO_4$, and concentrated in vacuo, affording 2-nitro-isophthalic acid diallyl ester (**23a**; 6.50 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.81 (d, J = 5.81 Hz, 4H) 5.33 (dd, J = 10.48, 1.14 Hz, 2H) 5.41 (dd, J = 17.18, 1.26 Hz, 2H) 5.97 (m, 2H) 7.67 (t, J = 7.83 Hz, 1H) 8.22 (d, J = 8.08 Hz, 2H).

A solution of **23a** (6.50 g, 22.3 mmol) in DMF (100 mL) was cooled to −78 °C. Methyl thioglycolate (2.02 mL, 22.3 mmol) was then added. The solution was allowed to stir for a few minutes at −78 °C, after which excess DBU was added (8.3 mL, 55.3 mmol). After 1 h, the reaction was judged complete by TLC. The reaction mixture was diluted with water (500 mL) and poured into a large Erlenmeyer flask. The desired product was precipitated out by adding 10% HCl (5–10 mL dropwise). The white solid was filtered, rinsed three times with water, and allowed to air-dry, affording 3-hydroxy-benzo[*b*]thiophene-2,7-carboxylic acid 7-allyl ester 2-methyl ester (**23b**) in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.98 (s, 3H) 4.94 (d, J = 5.81 Hz, 2H) 5.34 (dd, J = 10.48, 1.14 Hz, 1H) 5.48 (dd, J = 17.18, 1.26 Hz, 1H) 6.10 (m, 1H) 7.51 (t, J = 7.71 Hz, 1H) 8.16 (dd, J = 7.96, 1.14 Hz, 1H) 8.31 (dd, J = 7.45, 1.14 Hz, 1H) 10.08 (br s, 1H).

A solution of **23b** (1.0 g, 3.4 mmol) in DCM (20 mL) was cooled to 0 °C. Triethylamine (1.2 mL, 8.6 mmol) was then added, followed by mesyl chloride (0.28 mL, 3.6 mmol). The reaction mixture was allowed to stir at 0 °C for 30 min, after which it was judged complete by TLC. Excess water was then added, and the desired product was extracted into DCM (3× 25 mL). The organic layers were combined, washed with aqueous sodium bicarbonate, dried over $MgSO_4$, and concentrated in vacuo. The crude product was then dissolved in a minimal amount of DCM and filtered through a thick pad of silica gel using a 40% ethyl acetate/hexane solution, ultimately affording 3-methanesulfonyloxy-benzo[*b*]thiophene-2,7-carboxylic acid 7-allyl ester 2-methyl ester (**23c**; 1.0 g, 79%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.53 (s, 3 H) 3.96 (s, 3H) 4.95 (d, J = 5.81 Hz, 2H) 5.35 (dd, J = 10.36, 1.26 Hz, 1H) 5.48 (dd, J = 17.18, 1.52 Hz, 1H) 6.09 (m, 1H) 7.60 (t, 1H) 8.27 (dd, J = 8.08, 1.26 Hz, 1H) 8.32 (dd, J = 7.45, 1.14 Hz, 1H).

Following the procedure in the preparation of **23b**, **23c** (1.0 g, 2.7 mmol) was converted to 3-hydroxy-thieno[3,2-*b*][1]benzothiophene-2,5-dicarboxylic acid 5-allyl ester 2-methyl ester (**23d**) in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.97 (s, 3H) 4.96 (d, J = 5.81 Hz, 2H) 5.36 (dd, J = 10.61, 1.26 Hz, 1H) 5.49 (dd, J = 17.18, 1.26 Hz, 1H) 6.06–6.17 (m, 1H) 7.56 (t, J = 7.71 Hz, 1H) 8.08 (dd, J = 7.96, 1.14 Hz, 1H) 8.26 (dd, J = 7.58, 1.01 Hz, 1H) 10.04 (br s, 1H).

To a solution of **23d** (1.05 g, 3.02 mmol) in DMF (25 mL) were added excess potassium carbonate and ethyl bromoacetate (0.5 mL, 4.5 mmol). The reaction was allowed to stir at ambient temperature for 2 h after which it was judged complete by TLC. Excess water was added to precipitate the desired product 3-ethoxycar-

bonylmethoxy-thieno[3,2-*b*][1]benzo-thiophene-2,5-dicarboxylic acid 5-allyl ester 2-methyl ester (**23e**) as a white solid in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.31 (t, J = 7.20 Hz, 3H) 3.92 (s, 3H) 4.28 (q, J = 7.07 Hz, 2H) 4.95 (d, J = 5.56 Hz, 2H) 5.11 (s, 1H) 5.35 (dd, J = 10.36, 1.26 Hz, 1H) 5.48 (dd, J = 17.18, 1.52 Hz, 1H) 5.96–6.24 (m, 1H) 7.55 (t, J = 7.71 Hz, 1H) 8.07 (dd, J = 7.96, 1.14 Hz, 1H) 8.24 (dd, J = 7.58, 1.01 Hz, 1H).

To a solution of **23e** (1.21 g, 2.8 mmol) in anhydrous THF (50 mL) was added $Pd(PPh_3)_4$ (320 mg, 0.28 mmol) under N_2 flow. Concentrated acetic acid was then added (2.5 mL), and the mixture was allowed to stir at ambient temperature for 2 h, after which the reaction was judged complete by TLC. A 20% ethyl acetate/hexane solution was added to the reaction to precipitate out the desired product, 3-ethoxycarbonylmethoxy-thieno[3,2*b*][1]benzo-thiophene-2,5-dicarboxylic acid 2-methyl ester (**23f**; 0.76 g, 68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.29 (t, J = 7.07 Hz, 3H) 3.90 (s, 3H) 4.25 (q, J = 7.07 Hz, 2H) 5.26 (s, 2H) 7.72 (t, J = 7.71 Hz, 1H) 8.22 (dd, J = 7.58, 1.01 Hz, 1H) 8.43 (dd, J = 7.96, 0.88 Hz, 1H).

To a solution of **23f** (450 mg, 1.14 mmol) in toluene (7.5 mL) were added diphenylphosphoryl azide (0.37 mL, 1.71 mmol), excess triethyl amine (0.40 mL, 2.86 mmol), and *t*-butanol (0.75 mL). The solution was allowed to stir at ambient temperature for 2 h, after which the starting material was judged consumed by TLC. An additional 0.75 mL of *t*-butanol was then added and the reaction mixture was heated to 100 °C and allowed to stir for an additional 16 h, after which the reaction was quenched with excess water and extracted into DCM (3× 10 mL). The organic layers were combined, washed with water (3× 10 mL), dried with $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash chromatography using ethyl acetate/hexane (5–20% gradient) as eluent to afford 5- *tert*-butoxycarbonylamino-3-ethoxycarbonylmethoxy-thieno[3,2-*b*][1]benzo-thiophene-2-carboxylic acid methyl ester (**23g**; 535 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.28 (t, J = 7.20 Hz, 3H) 3.92 (s, 3H) 4.26 (q, J = 7.16 Hz, 2H) 5.06 (s, 2H) 6.41 (br s, 1H) 7.45 (t, J = 7.96 Hz, 1H) 7.61 (dd, J = 7.83, 1.01 Hz, 1H) 7.92 (d, J = 8.08 Hz, 1H).

A solution of **23g** (170 mg, 0.37 mmol) in DCM (6 mL) was cooled to 0 °C. Trifluoroacetic acid (1.2 mL) was then added, and the solution was allowed to warm to ambient temperature, stirring for a total of 4 h, after which the reaction was judged complete by TLC. The reaction mixture was concentrated in vacuo, dissolved in ethyl acetate, and washed with sodium bicarbonate (3× 10 mL). The organic layer was then concentrated in vacuo, affording 5-amino-3-ethoxycarbonylmethoxy-thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid methyl ester (**23h**) in a quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.30 (t, J = 7.20 Hz, 3H) 3.91 (s, 3H) 4.28 (q, J = 7.24 Hz, 2H) 5.06 (s, 2H) 6.89 (dd, J = 7.58, 1.01 Hz, 1H) 7.33 (t, J = 7.71 Hz, 1H) 7.42 (dd, J = 7.97, 0.88 Hz, 1H).

To a solution of **23h** (250 mg, 0.68 mmol) in DCE (6.5 mL) was added cyclohexane carboxaldehyde (0.082 mL, 0.68 mmol), $NaBH(OAc)_3$ (250 mg, 1.2 mmol). HOAc (0.05 mL, 0.82 mmol) was then added dropwise and the solution was allowed to stir at ambient temperature for 2 h after which the reaction was judged complete by TLC. The solution was filtered through silica gel, concentrated in vacuo and purified by flash chromatography to give 5-(cyclohexylmethyl-amino)-3-ethoxycarbonylmethoxy-thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid methyl ester (**23i**; 180 mg, 57%)$CDCl_3$) δ ppm 0.97–1.11 (m, 2H) 1.23–1.33 (m, 5H) 1.63–1.81 (m, 6H) 1.83–1.90 (m, 2H) 3.15 (d, J = 6.82 Hz, 2H) 3.91 (s, 3H) 4.27 (q, J = 7.07 Hz, 3H) 5.07 (s, 2H) 6.67 (dd, J = 7.83, 0.76 Hz, 1H) 7.26 (d, J = 7.83, 1.01 Hz, 1H) 7.34 (t, J = 7.83 1H).

Compound **23i** (30 mg, 0.065 mmol) was hydrolyzed according to the procedure in the preparation of **1** to give compound **23** (8.2 mg, 28%) as a green solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.94–1.10 (m, 2H) 1.17–1.35 (m, 3H) 1.63–1.82 (m, 4H) 1.85–1.96 (m, 2H) 3.11–3.18 (m, 2H) 5.11 (s, 2H) 5.87–5.96 (m, 1H) 6.73 (d, J = 7.83 Hz, 1H) 7.31 (d, J = 7.83 Hz, 1H) 7.38 (t, J = 7.83 Hz, 1H). HRMS: calcd for $C_{20}H_{21}NO_5S_2$ + H+, 420.09339; found (ESI-FTMS, $[M+H]^{1+}$), 420.0931.

5.2.24. 3-(Carboxymethoxy)-6-[(cyclohexylmethyl)-amino]thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid (24). To a solution of 3-hydroxy-benzo[*b*]thiophene-2,6-dicarboxylic acid 6-allyl ester 2-methyl ester (3.2 g, 11 mmol) in DCM (50 mL) were added triethylamine (1.83 mL, 13 mmol), trifluoromethanesulfonyl anhydride (2.2 mL, 13 mmol), and DMAP (67 mg, 0.55 mmol) at 0 °C. The temperature was allowed to rise to room temperate and the mixture was stirred for 4 h, washed with aqueous $NaHCO_3$, and dried over $MgSO_4$. The crude product was purified by flash chromatography eluted with ethyl acetate/hexane to give 3-trifluoromethanesulfonyloxy-benzo[*b*]thiophene-2,6-dicarboxylic acid 6-allyl ester 2-methyl ester (**24a**; 3.88 g, 84%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.02 (s, 3H) 4.86–4.93 (m, 2H) 5.34 (dd, J = 10.48, 1.14 , 1H) 5.41–5.50 (m, J = 17.18, 1.52 1H) 5.98–6.15 (m, 1 H) 7.90 (d, J = 8.59 Hz, 1H) 8.19 (dd, J = 8.72, 1.39 Hz, 1H) 8.59 (s, 1H).

To a solution of **24a** (3.8 g, 8.96 mmol) in DCM were added methyl thioglycolate (0.96 mL, 10.75 mmol) and triethylamine (3.75 mL, 26.88 mL) at 0 °C. The temperature was allowed to rise to room temperature. The mixture was stirred for 24 h and washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and purified by flash chromatography eluted with ethyl acetate/hexane to give 3-methoxycarbonylmethyl-sulfanyl-benzo[*b*]thiophene-2, 6-dicarboxylic acid 6-allyl ester 2-methyl ester (**24b**; 1.81 g, 53%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.51 (s, 3H) 4.00 (s, 3H) 4.84–4.96 (m, 2H) 5.28–5.39 (m, 1H) 5.39–5.51 (m, 1H) 5.96–6.21 (m, 1H) 8.15 (dd, J = 8.59, 1.52 Hz, 1H) 8.25 (dd, J = 8.59, 0.76 Hz, 1H) 8.59 (dd, J = 1.52, 0.76 Hz, 1H).

To a solution of **24b** (1.05 g, 2.76 mmol) in DMF was added DBU (0.83 mL, 5.53 mmol) at room temperature. The resultant mixture was stirred for 24 h. To this were added ethyl bromoacetate (0.92 mL, 8.28 mmol) and K_2CO_3 (1.14 g, 8.26 mmoL). The reaction mixture was stirred for additional 2 h, then aqueous NH_4Cl (200 mL) and water (300 mL) were added. The white precipitate was collected by filtration and washed with water to give 3-ethoxycarbonylmethoxy-thieno[3,2-*b*][1]benzothiophene-2,6-dicarboxylic acid 6-allyl ester 2-methyl ester (**24c**; 1.16 g, 97% two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.29 (t, J = 7.07 Hz, 3H) 4.27 (q, J = 7.24 Hz, 2H) 4.85–4.92 (m, 2H) 5.07 (s, 2H) 5.30–5.38 (m, 1H) 5.40–5.51 (m, 1H) 5.97–6.17 (m, 1H) 7.91 (d, J = 7.83 Hz, 1H) 8.13 (dd, J = 8.46, 1.39 Hz, 1H) 8.59 (d, J = 1.52 Hz, 1H).

To a suspension of **24c** (920 mg, 2.12 mmol) in THF (50 mL) was added $Pd(PPh_3)_4$ (240 mg, 0.21 mmol) under N_2, followed by addition of HOAc (5 mL). The resultant mixture was stirred at room temperature for 2 h, diluted with hexane/ethyl acetate (4:1), and the precipitate was collected by filtration to give 3-ethoxycarbonylmethoxy-thieno[3,2-*b*][1]benzo-thiophene-2,6-dicarboxylic acid 2-methyl ester (**24d**; 779 mg, 93%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.22 (t, J = 7.07 Hz, 4H) 3.87 (s, 3H) 4.19 (q, J = 7.07 Hz, 2H) 5.19 (s, 2H) 8.05 (dd, J = 8.34, 1.52 Hz, 1H) 8.19–8.26 (m, 1H) 8.75 (dd, J = 1.52, 0.76 Hz, 1H) 13.25 (s, 1H).

To a solution of **24d** (290 mg, 0.73 mmol) in toluene (5 mL) and *t*-BuOH (0.5 mL) were added diphenylphosphoryl azide (0.238 mL, 1.1 mmol) and triethylamine (0.205 mL) at room temperature. The reaction mixture was stirred for 30 min, extra 0.5 mL *t*-BuOH was added, and the reaction mixture was heated to 100 °C. After stirring at this temperature overnight, the mixture was partitioned between DCM and water, and extracted with DCM, dried over $MgSO_4$. The crude product was purified by flash chromatography eluted with ethyl acetate/DCM to give 6- *tert*-butoxycarbonylamino-3-ethoxycarbonylmethoxy-thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid methyl ester (**24e**; 195 mg, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.29 (t, J = 7.20 Hz, 3H) 1.54 (s, 9H) 3.90 (s, 3H) 4.27 (q, J = 7.07 Hz, 2H) 5.04 (s, 2H) 6.72 (s, 1H) 7.19 (dd, J = 8.84, 2.02 Hz, 1H) 7.71 (d, J = 8.59 Hz, 1H) 8.17 (s, 1 H).

To **24e** (0.433 g, 0.88 mmol) was added a mixture of 4.4 mL of 1.0 N HCl in ethyl acetate (4.4 mmol), and 1.1 mL methanol. The mixture was allowed to stir at room temperature for 12 h. The resulting suspension was filtered and the solids were dried under vacuum to give 6-amino-3-ethoxycarbonylmethoxy-thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid methyl ester HCl salt as an off-white solid (**24f**; 0.302 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.41 (s, 9H) 3.78–3.82 (m, J = 1.77 Hz, 3H) 4.94–5.12 (m, 1H) 5.13 (d, J = 7.33 Hz, 1H) 6.98 (d, J = 8.08 Hz, 1H) 7.37 (s, 1H) 7.85 (d, J = 9.09 Hz, 1H).

Compound **24f** (0.100 g, 0.23 mmol) and cyclohexane carboxaldehyde (0.03 mL, 0.23 mmol), and 20 mL of 1,2-dichloroethane were added to a 50 mL round-bottomed flask under nitrogen. $NaBH(OAc)_3$ (0.123 g, 0.58 mmol) was then added, and the mixture was allowed to stir at room temperature for 12 h. The mixture was then poured into 100 mL methylene chloride and washed with three 50 mL portions of saturated sodium bicarbonate solution. The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed in vacuo to give the crude product. This was purified by silica gel chromatography (10% ethyl acetate/hexane) to give 6-cyclohexylmethylamino-3-ethoxycarbonylmethoxy-thieno[3,2-*b*][1]benzo-thiophene-2-carboxylic acid methyl ester (**24g**; 0.053 g, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.81–1.10 (m, 3H) 1.13–1.36 (m, 3H) 1.47 (s, 9H) 1.53–1.90 (m, 5H) 3.02 (d, J = 6.82 Hz, 2H) 3.88 (s, 3H) 4.92 (s, 2H) 6.69 (dd, J = 8.72, 2.15 Hz, 1H) 6.90 (d, J = 2.02 Hz, 1H) 7.58 (d, J = 8.59 Hz, 1H).

Compound **24g** (0.053g, 0.11 mmol) was added to a 50 mL round-bottomed flask containing 10 mL THF. A solution of $LiOH{\cdot}H_2O$ (0.018 g, 0.43 mmol, 4.0 equiv) in 5 mL water was then added, and the resulting mixture was allowed to stir at room temperature overnight. The resulting solution was then acidified by the addition of excess 1.2 N HCl. This mixture was then extracted with three 25 mL portions of ethyl acetate. The organic layer was then washed with three 50 mL portions of water and one 50 mL portion of saturated sodium chloride solution. The organic layer was then dried over $MgSO_4$, filtered, and the solvent was removed in vacuo to give the crude product. This was then purified by reverse-phase HPLC under basic conditions. The desired product was isolated pure as the bis-triethylamine salt (**24**; 0.004 g, 6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.95–1.09 (m, 2H) 1.11–1.28 (m, 18H) 1.57– 1.94 (m, 9H) 2.85–2.94 (m, J = 4.55 Hz, 12H) 2.97–3.07 (m, 2H) 4.81 (s, 2H) 6.69 (d, J = 7.83 Hz, 1H) 6.92 (s, 1H) 7.58 (d, J = 8.59 Hz, 1H). HRMS: calcd for $C_{20}H_{21}NO_5S_2$ + H+, 420.09339; found (ESI-FTMS, $[M+H]^{1+}$), 420.094.

5.2.25. 3-(Carboxymethoxy)-5-(cyclohexylamino)-thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid (25). To a solution of **23h** (250 mg, 0.68 mmol) in DCE (6.5 mL) were added cyclohexanone (0.071 mL, 0.68 mmol) and $NaBH(OAc)_3$ (250 mg, 1.2 mmol). HOAc (0.05 mL, 0.82 mmol) was then added dropwise and the solution was allowed to stir at ambient temperature for 2 h after which the reaction was judged complete by TLC. The solution was filtered through silica gel, concentrated in vacuo, and purified by flash chromatography using a 0–15% ethyl acetate/hexane gradient, affording 5-cyclohexylamino-3-ethoxycarbonylmethoxy-thieno[3,2-*b*][1]benzo-thiophene-2-carboxylic acid methyl ester (**25a**; 180 mg, 59%) as a viscous yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.21–1.35 (m, 6H) 1.35–1.49 (m, 2H) 1.65–1.74 (m, 1H) 1.77–1.88 (m, 2H) 2.07–2.18 (m, 2H) 3.36–3.56 (m, 1H) 3.91 (s, 3H) 4.27 (q, J = 7.24 Hz, 2H) 5.07 (s, 2H) 6.70 (dd, J = 7.83, 1.01 Hz, 1H) 7.24 (dd, J = 7.83, 1.01, 1H) 7.32 (t, J = 7.83 Hz, 1H).

Compound **25a** (30 mg, 0.07 mmol) was hydrolyzed according to the procedure in the preparation of **1** to give compound **25** (20 mg, 71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.15–1.30 (m, 1H) 1.32–1.46 (m, 4H)

1.64–1.73 (m, 1H) 1.75–1.85 (m, 2H) 1.98–2.07 (m, 2H) 5.07 (br s, 2H) 5.42 (d, J = 8.59 Hz, 1H) 6.78 (d, J = 7.58 Hz, 1H) 7.30 (dd, J = 7.83, 1.01 Hz, 1H) 7.35 (t, J = 7.71 Hz, 1H). HRMS: calcd for $C_{19}H_{19}NO_5S_2$ + H+, 406.07774; found (ESI-FTMS, $[M+H]^{1+}$), 406.0771.

5.2.26. 3-(Carboxymethoxy)-6-(cyclohexylamino)-thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid (26). To a solution of **24e** (466 mg, 1.0 mmol) in DCM (15 mL) was added trifluoroacetic acid (2 mL). The resultant mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure. The crude product (**26a**) was re-dissolved in DCE (10 mL). To this were added cyclohexanone (0.156 mL, 1.5 mmol) and HOAc (0.088 mL, 1.5 mmol), followed by the addition of $NaBH(OAc)_3$ (424 mg, 2.0 mmol). The resultant mixture was stirred at room temperature overnight. The crude product was purified by flash chromatography eluted with hexane/ethyl acetate to give the desired product, 6-cyclohexylamino-3-ethoxycarbonylmethoxy-thieno[3,2-*b*][1]benzo-thiophene-2-carboxylic acid methyl ester (**26b**; 260 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.10–1.26 (m, 2H) 1.33–1.48 (m, 2H) 1.61–1.95 (m, 4H) 2.09 (dd, J = 12.88, 3.28 Hz, 2H) 3.26–3.41 (m, 1H) 3.88 (s, 3H) 4.26 (q, J = 7.07 Hz, 2H) 5.02 (s, 2H) 6.66 (dd, J = 8.72, 2.15 Hz, 1H) 6.91 (d, J = 2.27 Hz, 1H) 7.57 (d, J = 8.34 Hz, 1H).

Compound **26b** (23 mg, 0.05 mmol) was hydrolyzed according to the procedure in the preparation of **1** to give compound **26** (13.2 mg, 63%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.84–1.12 (m, 4H) 1.11–1.31 (m, 2H) 1.40–1.50 (m, 1H) 1.51–1.65 (m, 2H) 1.80 (dd, J = 13.14, 3.03 Hz, 2H) 4.18 (s, 2H) 5.67 (d, J = 7.83 Hz, 2H) 6.59 (dd, J = 8.84, 2.02 Hz, 1H) 6.88 (d, J = 2.02 Hz, 1H) 7.37 (d, J = 8.59 Hz, 1H).

5.2.27. 3-(Carboxymethoxy)-6-(tetrahydro-2*H*-pyran-4-ylamino)thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid (27). $NaBH(OAc)_3$ (90 mg, 0.42 mmol) was added to a mixture of **26a** (85 mg, 0.17 mmol), tetrahydropyran-4-one (24 μl, 0.2 mmol), and acetic acid (5 μL) in DCE (4 mL). The mixture was stirred at 50 °C for 17 h. DCM (20 mL) was added and the mixture was washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtered, and rotovaped. Purification by flash chromatography yielded 3-ethoxycarbonylmethoxy-6-(tetrahydro-pyran-4-ylamino)-thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid methyl ester (**27a**; 72 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.29 (t, J = 7.2 Hz, 3H), 1.44–1.61 (m, 3H), 2.08 (d, J = 14.4 Hz, 2H), 3.44–3.70 (m, 3H), 3.88 (s, 3H), 3.98–4.09 (m, 2H), 4.27 (q, J = 7.2 Hz, 2H), 5.02 (s, 2H), 6.71 (dd, J = 8.6, 1.8 Hz, 1H), 6.96 (s, 1H), 7.61 (d, J = 8.6 Hz, 1H).

Compound **27a** (72 mg, 0.16 mmol) was hydrolyzed according to the procedure in the preparation of **1** to give compound **27** (65 mg, 77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.46–1.92 (m, 2H) 1.92 (d, J = 13.39 Hz, 2H) 3.88 (m, J = 11.12 Hz, 1H) 4.83 (s, 1H) 6.18 (d, J = 7.83 Hz, 1H) 6.82 (dd, J = 8.84, 2.02 Hz, 1H) 7.15 (d, J = 2.02 Hz, 1H) 7.68 (d, J = 8.84 Hz, 1H). HRMS: calcd for $C_{18}H_{17}NO_6S_2$ + H+, 408.05701; found (ESI+, $[M+H]^{1+}$), 408.05609.

5.2.28. 3-(Carboxymethoxy)-6-{[1-(ethylsulfonyl)-piperidin-4-yl]amino}thieno[3,2-*b*][1]benzo-thiophene-2-carboxylic acid (28). $NaBH(OAc)_3$ (227 mg, 1.07 mmol) was added to a mixture of **26a** (259 mg, 0.54 mmol), 4-oxopiperidine-1-carboxylic acid *tert*-butyl ester (129 mg, 0.65 mmol), and acetic acid (50 μL) in DCE (4 mL). The mixture was stirred at 50 °C for 17 h. DCM (20 mL) was added and the mixture was washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtered, and evaporated. Purification by flash chromatography yielded 4-(3-ethoxycarbonylmethoxy-2-methoxycarbonyl-thieno[3,2-*b*][1] benzothiophen-6-ylamino)-piperidine-1-carboxylic acid *tert*-butyl ester (**28a**; 237 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.29 (t, J = 7.2 Hz, 3H), 1.34–1.44 (m, 2H), 1.47 (s, 9H), 1.79–1.91 (m, 1H), 2.08 (d, J = 12.1 Hz, 2H), 2.87–3.10 (m, 3H), 3.43–3.58 (m, 1H), 3.78–3.94 (m, 5H), 4.00–4.16 (m, 2H), 4.26 (q, J = 7.1 Hz, 2H), 5.02 (s, 2H), 6.69 (dd, J = 8.6, 2.0 Hz, 1H), 6.94 (d, J = 2.0 Hz, 1H), 7.60 (d, J = 8.6 Hz, 1H).

Compound **28a** (204 mg, 0.37 mmol) was stirred in a mixture of trifluoroacetic acid (10 mL) and DCM (10 mL) at room temperature for 1 h. Evaporation of solvent gave a trifluoroacetic acid salt of 3-ethoxycarbonylmethoxy-6-(piperidin-4-ylamino)-thieno[3,2-*b*][1]-benzothiophene-2-carboxylic acid methyl ester (**28b**; 206 mg, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.21 (t, J = 7.1 Hz, 2H), 1.50–1.64 (m, 2H), 2.04–2.15 (m, 2H), 2.96–3.09 (m, 2H), 3.11–3.23 (m, 1H), 3.28–3.40 (m, 2H), 3.57–3.68 (m, 1H), 3.79 (s, 2H), 4.17 (q, J = 7.1 Hz, 2H), 5.11 (s, 2 H), 6.84 (dd, J = 8.8, 2.0 Hz, 1H), 7.17 (d, J = 2.0 Hz, 1H), 7.75 (d, J = 8.6 Hz, 1H), 8.22–8.54 (m, 2H).

Compound **28b** (83 mg, 0.15 mmol) was treated with ethylsulfonyl chloride (14 μl, 0.15 mmol) in a mixture of DCM (3 mL) and saturated $NaHCO_3$ (2 mL). The reaction was monitored by LCMS to completion, DCM was then added, and the organic layer was washed with water, brine, dried over $MgSO_4$, and filtered. Evaporation of solvent yielded 6-(1-ethanesulfonyl-piperidin-4-ylamino)-3-ethoxycarbonylmethoxy-thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid methyl ester (**28c**; 58 mg, 73% two steps) as a green film. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.29 (t, J = 7.1 Hz, 3H), 1.39 (t, J = 7.5 Hz, 3H), 1.56–1.65 (m, 3H), 2.15–2.28 (m, J = 12.3, 2.9 Hz, 2H), 2.94–3.09 (m, 4H), 3.44–3.59 (m, 1H), 3.79–3.86 (m, 2H), 3.88 (s, 3H), 4.26 (q, J = 7.1 Hz, 2H), 5.02 (s, 2H), 6.70 (dd, J = 8.6, 2.0 Hz, 1H), 6.94 (d, J = 2.0 Hz, 1H), 7.62 (d, J = 8.6 Hz, 1H).

Compound **28c** (58 mg, 0.11 mmol) was hydrolyzed according to the procedure in the preparation of **1** to give compound **28** (38 mg, 72%) as a golden yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.23 (t, J = 7.33 Hz, 3H) 1.48–1.35 (m, 2H) 2.06–1.97 (m, 2H) 3.05–2.96 (m, 2H) 3.07 (q, J = 7.41 Hz, 2H) 3.60 (d, J = 12.63 Hz, 2H) 4.55–4.31 (m, 2H) 6.20–5.95 (m, 2H) 6.80 (d, J = 8.84 Hz, 1H) 7.14 (s, 1 H) 7.73–7.56 (m, 1H). HRMS: calcd for $C_{20}H_{22}N_2O_7S_3-H+$, 497.05164; found (ESI-FTMS, $[M-H]^{1-}$), 497.0527.

5.2.29. 6-{[1-(Benzylsulfonyl)piperidin-4-yl]amino}-3-(carboxymethoxy)thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid (29). Compound **28b** (65 mg, 0.12 mmol) was treated with α-tolylsulfonyl chloride (23 mg, 0.12 mmol) in a mixture of DCM (2 mL) and saturated $NaHCO_3$ (2 mL). The reaction was monitored by LCMS to completion, DCM was then added, and the organic layer was washed with water, brine, dried over $MgSO_4$, and filtered. Evaporation of solvent yielded 3-ethoxycarbonylmethoxy-6-(1-phenylmethanesulfonyl-piperidin-4-ylamino)-thieno[3,2-*b*][1]benzothiophene-2-carboxylic acid methyl ester (**29a**; 37 mg, 53% two steps) as a green solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.29 (t, J = 7.2 Hz, 3H), 1.35–1.50 (m, 2H), 1.99–2.12 (m, J = 13.4 Hz, 2H), 2.71–2.86 (m, 2H), 3.33–3.47 (m, 1H), 3.59– 3.69 (m, 2H), 3.74–3.82 (m, J = 8.6 Hz, 1H), 3.88 (s, 3H), 4.21–4.31 (m, 4H), 5.02 (s, 2H), 6.66 (dd, J = 8.7, 2.1 Hz, 1H), 6.89 (d, J = 2.3 Hz, 1H), 7.37–7.46 (m, 5H), 7.60 (d, J = 8.6 Hz, 1H).

Compound **29a** (37 mg, 0.06 mmol) was hydrolyzed according to the procedure in the preparation of **1** to give compound **29** (22 mg, 65%) as a green solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.24 (s, 1H) 1.43–1.29 (m, 2H) 1.96 (s, 1H) 2.92 (t, J = 10.74 Hz, 2H) 3.55 (d, J = 12.38 Hz, 1H) 4.42 (s, 2H) 4.56 (s, 2H) 6.14 (d, J = 8.08 Hz, 1H) 6.80 (dd, J = 8.59, 2.02 Hz, 1H) 7.15 (d, J = 1.77 Hz, 1H) 7.45–7.36 (m, 5H) 7.67 (d, J = 8.59 Hz, 1H). HRMS: calcd for $C_{25}H_{24}N_2O_7S_3$ + H+, 561.08184; found (ESI+, $[M+H]^{1+}$), 561.08092.

5.3. Enzymatic assay

The enzymatic assay was carried out at room temperature in 96-well plates. The assay buffer contained 50 mM of 3,3-dimethyl glutarate, 1 mM EDTA, 1 mM TCEP, and 0.01% Triton (pH 7.0 with an ionic strength of 0.15 M adjusted by sodium chloride). The reaction was initiated by addition of the enzyme at a final concentration of 10 or 100 nm for PTP1B, 20 nm for TCPTP, 20 nM for CD45, and 270 nM for LAR, respectively. The initial rate of PTPase-catalyzed hydrolysis of *p*-nitrophenol phosphate (pNPP) was measured by following the absorbance change at 405 nm. IC_{50} value was determined under fixed pNPP concentration of 1 mM. All the assays were carried out in duplicate or triplicate and the average results are presented. K_i is derived from IC_{50} based on competitive inhibition $K_i = IC_{50} \times K_m/(K_m + [\text{substrate}])$. PTPases used in the assays were recombinant human PTP1B (hPTP1B catalytic domain, residues 1–299, expressed and purified according to literature procedures[21]), recombinant human TCPTP (residues 1–299, expressed and purified in-house), recombinant human CD45 (cytoplasmic domain, residues 484–1281, purchased from Biomol), and recombinant human LAR (soluble catalytic LAR-D1 domain, residues 1275–1613, purchased from Biomol).

5.4. X-ray crystallographic studies

Human recombinant PTP1B catalytic domain (residues 1–299) was prepared as described.[21] The complex crystals were obtained by a similar method as described previously.[36] Diffraction data of the hPTP1B-inhibitor complex crystals were collected by in-house conventional X-ray generator using Raxis-IV image detectors. All data were collected at 100 K and processed with DENZO/SCALPACK.[37]

The structure was solved by molecular replacement with the available hPTP1B catalytic domain structure model. Cycles of model rebuilding and refinement were carried out with CNS (Brunger, A.T. and et. al. copyright: 1997–2001, Yale University), a system for X-ray crystallography and NMR and QUANTA (Accelrys Inc.). Inhibitor was built into the difference density after the first cycle of refinement. Water molecules were assigned according to Fo-Fc maps. The final model for hPTP1B-compound **1** contained residues 2–298, 128 waters, and 1 inhibitor. It exhibited good stereochemistry, with an averaged bond length and bond angle deviation from ideal geometry of 0.007 Å and 1.367°, respectively. The overall R factor is 22.3%, and R free is 24.9% with 5% test reflections, using diffraction data between 20 and 2.0 Å. The hPTP1B-compound **29** structure contained residues 2–298, 171 waters, and 1 inhibitor. The averaged bond length and bond angle deviation from ideal geometry are 0.01 Å and 1.65°, respectively. The overall R factor is 21.5%, and R free is 24.9% with 5% test reflections, using diffraction data between 20 and 2.1 Å. No residues were in disallowed regions of the Ramachandran plot for both structures.

5.5. Molecular modeling

Analogs were initially docked into the active site of the PTP1B protein structure from the X-ray complex with compound **1**. As additional protein–inhibitor complex structures were determined the binding site model was refined. Typically 1000 Monte Carlo cycles[38] were carried out to dock each analog into the site, while also allowing select protein residues to undergo constrained movement. Figures 2, 4, and 6 were generated using PyMOL (DeLano Scientific, 2002, San Carlos, CA, USA).

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