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# Novel thiophene derivatives as PTP1B inhibitors with selectivity and cellular activity

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ABSTRACT

A series of novel thiophene derivatives was designed, synthesized and their activities as competitive inhibitors of protein tyrosine phosphatase (PTPs) 1B (PTP1B) inhibitors were evaluated. All the compounds showed inhibitory potencies, and 10 of these exhibited moderate inhibitory activities with $IC_{50}$ values less than 10 μM. The activity of the most potent compound **P28** ($IC_{50}$ = 2.1 μM) was 15 times higher than that of the hit compound **P01**. Further, four representative compounds ( **P19, P22, P28**, and **P31**) demonstrated remarkably high selectivities against other PTPs (e.g., PTPα, LAR, CD45, and TCPTP); **P19** exhibited greater than sixfold selectivity over highly homologous TCPTP. More importantly, these compounds are permeable to cell membranes. The treatment of CHO-K1 cells with **P28** (10 μM) resulted in increased phosphorylation of AKT, which suggested extensive cellular activity of this compound. The novel chemical entities reported in this study could be used for overcoming the poor selectivity and low cellular activity of PTP1B inhibitors and might represent a starting point for development of therapeutic PTP inhibitors.



## 1. Introduction

Protein tyrosine phosphatase 1B (PTP1B), an intracellular protein tyrosine phosphatase (PTPs), has been implicated in the negative regulation of insulin and leptin signal transduction pathways among other biological functions.[1] Mice lacking functional PTP1B exhibit increased insulin sensitivity and improved glycemic control, and are resistant to diet-induced obesity.[2] Treatment of diabetic mice with PTP1B antisense oligonucleotides resulted in reduced PTP1B expression, and subsequent decreases in the adipose tissue mass, plasma insulin, and blood glucose levels.[3] It has been suggested that PTP1B inhibitors may be used for designing drugs for the treatment of type II diabetes and obesity. Moreover, since PTP1B has been reported to function as an oncogene in breast cancer, PTP1B-specific inhibitors can be used for effective treatment of breast cancer.[4]

Given the compelling evidence that PTP1B is associated with numerous human diseases, a large number of PTP1B inhibitors have been developed over the last decade with the aim of developing potent and selective compounds as drug candidates.[5] Shen et al.[5a] used a focused library approach to identify a non-hydrolyzable bisdifluorophosphonate (compound **1**, Fig. 1) which is the most potent compound to date; Liu et al.[5b] employed a linked-fragment approach to identify compound **2**, with a less polar phosphotyrosine (pTyr) mimetic and a more rigid linker, which displayed a 30-fold selectivity compared with TCPTP; Wiesmann et al.[5c] were the first to discover a series of allosteric inhibitors (compound **3** as the representative); these inhibitors might be used for developing selective inhibitors with acceptable pharmacological properties. Most of the reported compounds containing negatively-charged non-hydrolyzable pTyr mimetics, have exhibited excellent potency (at nanomolar concentrations) in vitro studies; however, the low cell permeability and low bioavailability of these compounds have limited their application for the development of effective drugs.[6] Thus, new potential drug candidates with improved physicochemical properties and bioavailability need to be developed.[7] Very recently, a selective noncompetitive PTP1B inhibitor, namely, trodusquemine,[8] developed by Genaera company, has proceeded to the phase I clinical trials. The results of the preclinical and early clinical trials were promising, and trodusquemine has been shown to possess both appetite suppressing effects and a hypoglycemic and hypocholesterolemic properties.

Compounds with a thiophene core have been widely studied due to their ability to bind to many receptors with high affinity.

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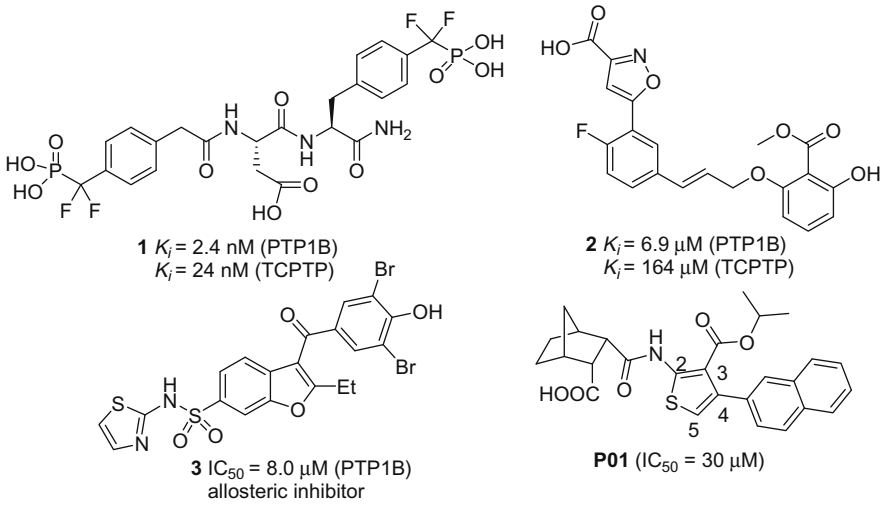



**Figure 1.** Structures of reported PTP1B inhibitors and the screening hit **P01**.

Recently, Wyeth pharmaceuticals have performed structure-based optimization and identified a series of monocyclic, bicyclic, and tricyclic thiophenes that can be used as highly potent PTP1B inhibitors.[9,6b] In the present study, we performed the in vitro screening of abundant substituted thiophene compounds from our in-house collection, and identified **P01** as a competitive PTP1B inhibitor, with an $IC_{50}$ value of 30 μM (Fig. S1, see in ESI). Molecular modeling suggested that **P01** simultaneously binds to the active site and the second pTyr binding site of PTP1B (Fig. S2). Further optimization of **P01** by extension of the molecule from the enzyme active site into the second pTyr binding site was explored to identification of novel moderately potent, selective, and cellular active PTP1B inhibitors.

## 2. Chemistry

### 2.1. Design of substituted thiophene derivatives

Based on the structure of thiophene derivative **P01** and molecule docking model of **P01** with PTP1B, 31 new thiophene-based analogues (**P02**–**P32**) (Table 1) were designed and synthesized for the first round. Replacing the cyclic acid moiety in **P01** with other cyclic acids or acyclic acids, we obtained analogues **P02**–**P06**. Removal or esterification of the cyclic acid led to compounds **P07** and **P08**. Keeping the 2-naphthalenyl ring, we changed the *i*-propyl ester for different alkyl ester, and obtained compounds **P09**–**P12**. Replacement of the 2-naphthalenyl ring with the phenyl ring and substituted phenyl ring led to five compounds (**P13**–**P17**). By cycling the $R_3$ and $R_4$ group in thiophene ring with $-(CH_2)_4-$ group, we got **P18**. Compounds **P19**–**P21** were obtained by substituting the 2-naphthalenyl with biphenyl-4-yl group. On the basis of the structure features of compounds **P19** and **P21**, we designed analogues **P22**–**P28** via introduction of various electronic, hydrophobic groups to the *ortho*-, *meta*-, or *para*-position of the terminal phenyl ring of biphenyl-4-yl group. Displacing the biphenyl-4-yl group of **P21** to biphenyl-3-yl or biphenyl-2-yl group, we afforded compounds **P29** and **P30**. The introduction of a methyl group to 5-position in thiophene ring of **P21** led to compound **P31**. At last, moving the phenyl group from $R_3$ to $R_4$ in compound **P13**, we obtained analogue **P32**.

### 2.2. Synthesis of substituted thiophene derivatives

Scheme 1 describes the straightforward synthesis of the analogues **P01–P32**. The substituted 2-aminothiophene intermediates **5** were prepared from simple ketones by microwave-assisted Gewald's reaction in a solvent-free medium, which was optimized previously.[10] Direct reaction of **5** with benzoyl chloride afforded compound **P07**. We performed overnight refluxing of **5** with a variety of acid anhydrides in dry $CH_2Cl_2$ under Ar and obtained compound **6** (**P01–P06**, **P09–P22**, and **P32**) in moderate to high yields. Compound **P21** was esterified with MeOH to yield **P08**. Finally, **P23–P28** with substituted biphenyl-4-yl moieties and compounds **P29–P31** were obtained in excellent yields using the microwave-assisted Suzuki coupling reaction of halogenated benzene derivatives **7** with boronic acid derivatives in $H_2O$.[11]

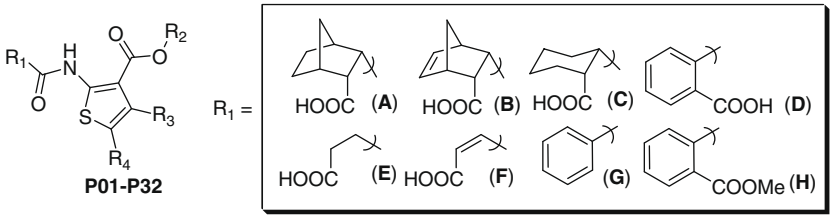
## 3. Results and discussion

### 3.1. PTP1B inhibitory activities and structure–activity relationship

For the primary assay, we measured the percentage inhibitory rates of all the synthetic compounds at concentrations of 100 and 10 μM. Compounds with good inhibition rates (>50% at 10 μM) were selected for further determination of $IC_{50}$ values (Table 1). The binding mode of **P01** to PTP1B revealed the orientation of the cyclic acid moiety, which is located deep inside the active site (Fig. S2). A slightly improving potency was observed when the bicycle[2.2.1]heptane-2-carboxylic acid moiety in **P01** was replaced with other cyclic acid moieties, such as bicyclo[2.2.1]hept-5-ene-2-carboxylic acid (**P02**), 1,2-cyclohexanedicarboxylic acid (**P03**), or 1,2-benzenedicarboxylic acid (**P04**). In contrast, the potency was remarkably decreased if acyclic acid moieties were used (**P05** vs **P01–P04** and **P06** vs **P19–P21**). As expected, the removal or esterification of the carboxylic acid in compound **P21** resulted in a loss of activity (e.g., **P07** and **P08**). This indicated that cyclic acid moiety favors the maintenance of the inhibitory activity. A comparison of the potencies of compounds **P03**, **P09**, and **P10** suggested that a smaller alkyl ester group (Me, Et, and *i*-Pr) in the 3-thiophenencarboxylic acid moiety did not change the potency against the PTP1B enzyme to remarkable extent, whereas, the incorporation of a bulky benzyl ester in the 3-thiophenencarboxylic acid moiety, as in the case of compound **P11**, decreased the activity of the compound.

Furthermore, the 2-naphthalenyl ring of **P01** can pass through the 'gateway' between the active site and the second pTyr binding site of PTP1B, and extend to the second binding site as shown in Figure S2. The comparison of the compounds **P13–P21** suggested that the inhibitory activity was affected greatly by the substituents

**Table 1**
Structures and inhibition of PTP1B of thiophene derivatives **P01–P32**




| Compd | $R_1$ | $R_2$ | $R_3$ | $R_4$ | % Inhibition at 100 μM | % Inhibition at 10 μM | $IC_{50}$[a] (μM) |
|---|---|---|---|---|---|---|---|
| **P01** | A | *i*-Pr | 2-Naphthenyl | H | 67.0 | 3.5 | 30.0 |
| **P02** | B | *i*-Pr | 2-Naphthenyl | H | 98.7 | 6.8 | —[b] |
| **P03** | C | *i*-Pr | 2-Naphthenyl | H | 100 | 16.0 | — |
| **P04** | D | *i*-Pr | 2-Naphthenyl | H | 100 | 23.3 | — |
| **P05** | E | *i*-Pr | 2-Naphthenyl | H | 21.5 | — | — |
| **P06** | F | *i*-Pr | Biphenyl-4-yl | H | 98.4 | 16.7 | — |
| **P07** | G | *i*-Pr | Biphenyl-4-yl | H | 18.7 | — | — |
| **P08** | H | *i*-Pr | Biphenyl-4-yl | H | 19.2 | — | — |
| **P09** | C | Et | 2-Naphthenyl | H | 96.4 | 20.4 | — |
| **P10** | C | Me | 2-Naphthenyl | H | 92.6 | 15.4 | — |
| **P11** | C | Bn | 2-Naphthenyl | H | 72.1 | — | — |
| **P12** | B | Et | 2-Naphthenyl | H | 95.9 | 2.4 | — |
| **P13** | B | Et | Phenyl | H | 10.7 | — | — |
| **P14** | C | *i*-Pr | 4-$NO_2$-phenyl | H | 45.0 | — | — |
| **P15** | B | Et | 4-F-phenyl | H | 30.0 | — | — |
| **P16** | D | *i*-Pr | Phenyl | H | 26.6 | — | — |
| **P17** | D | Et | 4-Br-phenyl | H | 97.3 | 3.4 | — |
| **P18** | B | *i*-Pr | $-(CH_2)_4-$ | | 27.5 | — | — |
| **P19** | B | *i*-Pr | Biphenyl-4-yl | H | 90.6 | 63.2 | 6.7 |
| **P20** | C | *i*-Pr | Biphenyl-4-yl | H | 84.4 | 23.4 | — |
| **P21** | D | *i*-Pr | Biphenyl-4-yl | H | 99.5 | 91.8 | 5.4 |
| **P22** | B | *i*-Pr | 4′-Cl-biphenyl-4-yl | H | 90.5 | 62.0 | 6.8 |
| **P23** | D | Et | 2′-MeO-biphenyl-4-yl | H | 100.0 | 61.3 | 8.5 |
| **P24** | D | Et | 3′-MeO-biphenyl-4-yl | H | 99.4 | 95.6 | 4.8 |
| **P25** | D | Et | 4′-MeO-biphenyl-4-yl | H | 99.2 | 92.3 | 4.6 |
| **P26** | D | Et | 4′-F-biphenyl-4-yl | H | 100.0 | 61.6 | 5.0 |
| **P27** | D | Et | 4′-$CF_3$-biphenyl-4-yl | H | 86.3 | 24.9 | — |
| **P28** | D | Et | 3′-Cl-4′-F-biphenyl-4-yl | H | 99.3 | 95.3 | 2.1 |
| **P29** | D | *i*-Pr | Biphenyl-3-yl | H | 98.6 | 84.7 | 8.7 |
| **P30** | D | Et | Biphenyl-2-yl | H | 96.6 | 2.0 | — |
| **P31** | D | Et | Biphenyl-4-yl | Me | 100.0 | 95.3 | 3.2 |
| **P32** | B | *i*-Pr | H | Ph | 15.5 | — | — |
| **3**[c] | | | | | 99.4 | 79.5 | 3.0 |

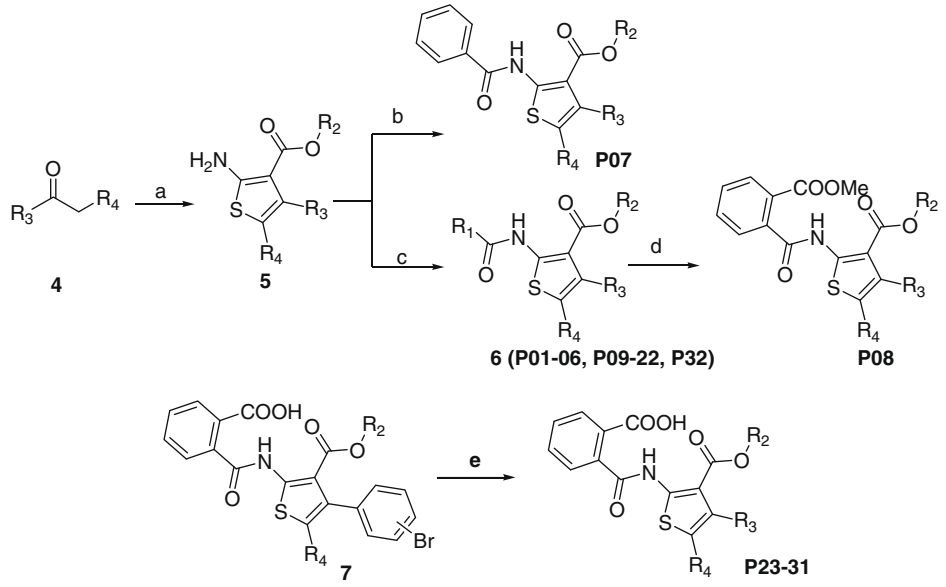
[a] Values are means of three determinations and deviation from the mean is <10% of the mean value.
[b] '—': The $IC_{50}$ value or inhibitory rate at 10 μM was not determined.
[c] **3**: Positive control.

on 4-position of the thiophene template. The introduction of rings smaller than the 2-naphthalenyl ring, such as simple phenyl ring (e.g., **P13** and **P16**) or substituted phenyl ring (e.g., **P14**, **P15**, and **P17**), resulted in decrease or loss of potency. Conversely, when a biphenyl-4-yl group was introduced, a remarkable enhancement in activity was achieved (**P21** vs **P04**). The increase in potency maybe ascribed to the length of the biphenyl-4-yl group that extends toward Met258 and forms a stronger van der Waals interaction with its side chain.

In order to optimize van der Waals interaction between the compound and the Met258 side chain of PTP1B, various electronic and hydrophobic groups were introduced at the *ortho*-, *meta*- or *para*-positions of the unsubstituted phenyl ring of biphenyl-4-yl group to afford compounds **P22–P28**. It was observed that compounds with *meta*- or *para*-methoxyl substituents were more potent than those with *ortho*-methoxyl substituents (**P23–P25**). The introduction of a chloro- or fluoro-group at the *para*-position (**P22** and **P26**) did not translate into better inhibitory activity, whereas simultaneous introduction of the chloro- and fluoro-groups at the *meta*- and *para*-positions, respectively, afforded the most potent compound **P28** with an $IC_{50}$ value of 2.1 μM, which is ~15-fold more potent than the initial hit compound **P01**. Conversely, compound **P27** containing the electron-withdrawing group of *para*-$CF_3$ exhibited a remarkably reduced in enzyme potency. While the compound **P29** with a biphenyl-3-yl group was approximately equipotent to the biphenyl-4-yl group substituted compound, compound **P30** with a biphenyl-2-yl substituent was less active. Finally, we also found that a small substituent like Me on the 5-position of the thiophene scaffold is beneficial for improving potency (**P31**). The structure–activity relationships (SAR) are summarized in Figure 2.

### 3.2. Selectivity against other phosphatases

In addition to potency improvements, we investigated the selectivity of four representative compounds, namely, **P19, P22, P28**, and **P31** against other PTPs (TCPTP, CD45, PTPα, and LAR). As shown in Figure 3, all the four compounds demonstrated excellent selectivity against PTPα and LAR (>10-fold) and moderate selectivity against CD45 (~3–5-fold). The differences in the selectivities of PTP1B and CD45 may be partially attributed to the differences between the active site and the second phosphotyrosine




**Scheme 1.** Synthesis of compounds **P01–P32**. Reagents and conditions: (a) $CNCH_2COOR_2$, S, $CH_3COONH_4$, morpholine, basic $Al_2O_3$, MW, 320 W, 10 min; (b) PhCOCl, $CH_2Cl_2$, TEA, rt; (c) acid anhydride, $CH_2Cl_2$, Ar, reflux; (d) MeOH, $H_2SO_4$, reflux; (e) $ArB(OH)_2$, $Na_2CO_3$, TBAB, $Pd(PPh_3)_4$, $H_2O$, MW, 150 °C, 10 min.

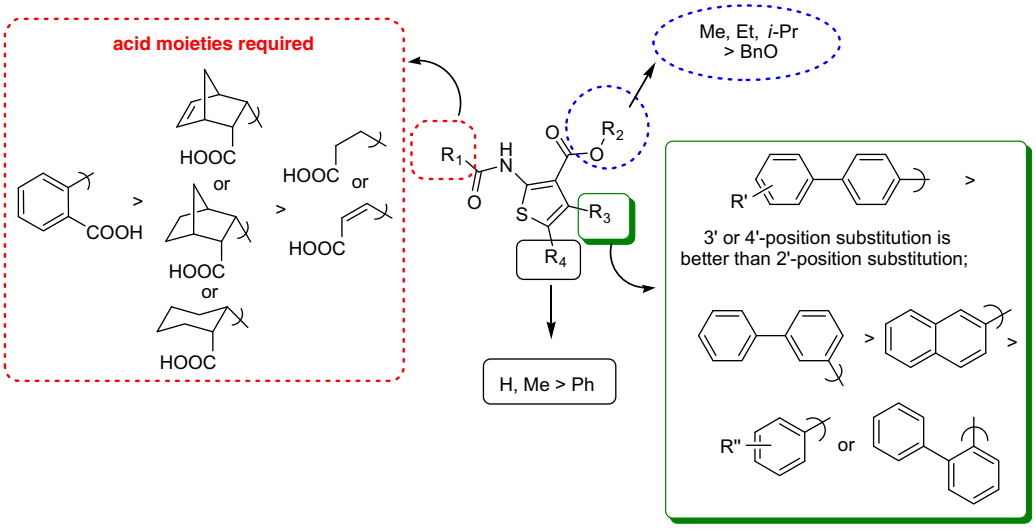



**Figure 2.** Structure–activity relationships.

binding site at the gateway of these two enzymes.[13] PTP1B has a glycine (Gly259) lining the bottom of the gateway, while CD45 has a larger amino acid, leucine. Among these four compounds, **P19** showed greater than sixfold selectivity against TCPTP, the most homologous PTPs, which is comparable with that of allosteric inhibitor **3**. Further, the selectivity of **P28** and **P31** (obtained by replacing the bicycle[2.2.1]hept-5-ene-2-carboxylic acid moiety in **P19** with 1,2-benzenedicarboxylic acid) against TCPTP was lower (~2-fold) than that of **P19**; the introduction of a 4′-Cl (**P22**) at the biphenyl-4-yl group of **P19** to function at the second pTyr binding site did not translate into better selectivity against TCPTP.

### 3.3. Cellular activity of selected compound

Phosphatidylinositol 3-kinase/AKT (PI3 K/AKT) signaling is known to be the major pathway affecting insulin-stimulated

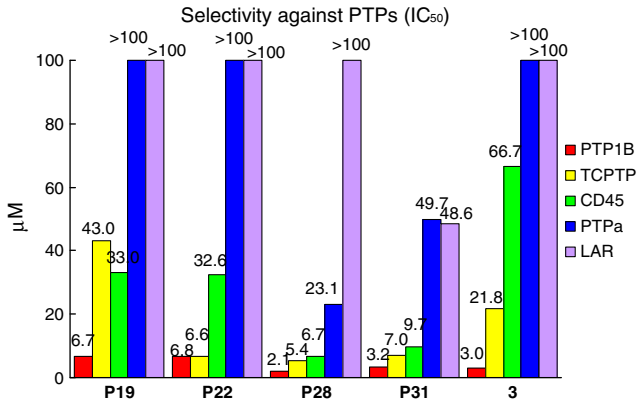



**Figure 3.** The $IC_{50}$ values of compounds **P19**, **P22**, **P28**, and **P31** against PTPs.

glucose metabolism. PTP1B inhibitors have affected the activation of AKT in CHO-K1 cells.[7b,14] Hence, we next explored the possible regulatory role of our most potent compound **P28** in AKT activation. As shown in Figure 4, the treatment of the CHO-K1 cells with compound **P28** (10 μM) resulted in an expected increase in the phosphorylation of AKT, which is consistent with the result of the known PTP1B inhibitor **3**. These results suggest that **P28** possesses good membrane permeability, and could possibly induce insulin signaling at the cellular level.

### 3.4. Binding models

To obtain structural information for further structural optimization studies, we constructed the 3D binding models of compound **P28** to PTP1B in open conformation based on the docking simulation, as shown in Figure 5. Similar to the binding model of **P01**, the model of **P28** revealed a dense network of hydrogen bonds between the carboxylic acid group of **P28** and Ala217, Cys215, Ser216, and Arg221 at the active site of PTP1B was observed as well as hydrophobic interactions between the phenyl ring of the ligand and the surrounding amino acid side chains (e.g., Asn111, Lys120).[15] Tyr46 was anticipated to establish aromatic stacking interactions with the thiophene scaffold which are commonly observed in many known PTP1B/inhibitor complexes.[16,5b,6b,7a] An additional interaction through hydrogen bond between Tyr46 and the amide oxygen of compound **P28** was possible. Furthermore, **P28** fitted deeply into the second pTyr binding site and established favorable hydrophobic interactions with the lipophilic amino acid side chains (Met258, Gly259, and Phe52) of PTP1B. Because of these multiple interactions, **P28** positioned itself effectively at the enzyme active site to serve as a potent inhibitor.

## 4. Conclusion

In conclusion, a series of novel thiophene derivatives were designed and synthesized and their potency as competitive PTP1B inhibitors was evaluated. After performing systematic SAR studies of $R_1$–$R_4$ substituents of the screen hit **P01**, we identified 10 compounds with $IC_{50}$ values less than 10 μM. Among these, four representative compounds ( **P19, P22, P28**, and **P31**) demonstrated excellent selectivity against PTPα and LAR (>10-fold), and moderate selectivity against CD45 (~3–5-fold). More importantly, **P19** exhibited greater than sixfold selectivity over TCPTP. Further studies on cellular activities revealed that **P28** was membrane-permeable and exerted extensive cellular effects on the activation of PI3K/AKT pathway in CHO-K1 cells. The novel chemical entities reported in this study could provide a possible opportunity for developing novel PTP1B inhibitors with promising selectivity and improved pharmacological properties.

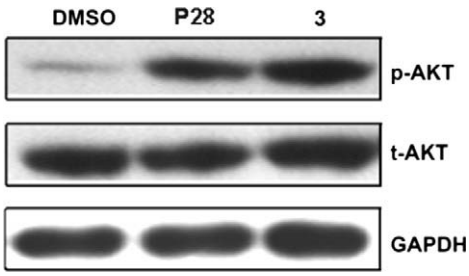



**Figure 4.** **P28** significantly activated phosphorylation of AKT in CHO-K1 cells. CHO-K1 cells were serum-starved for 2 h and then incubated with 10 μM **P28** or **3** for 2 h. Cells were lysed and immuno-blotted with indicated antibody. GAPDH was used as loading control.

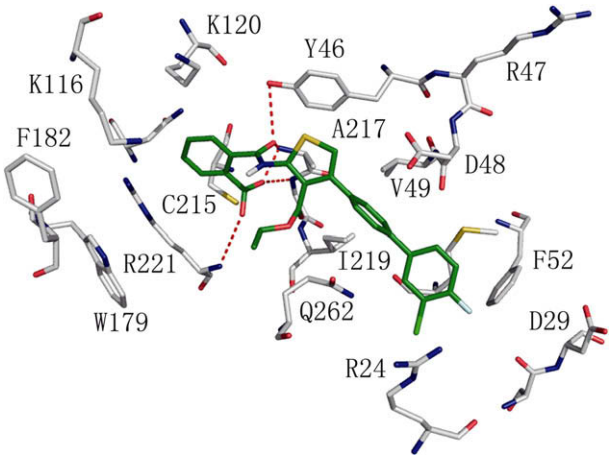



**Figure 5.** Binding mode of **P28** (**B**) to PTP1B derived from docking simulations. The inhibitor **P28** and key residues of PTP1B are shown in a capped stick representation. Carbon is in green for ligands and gray for PTP1B, oxygen is in red, nitrogen is in blue, sulfur is in yellow. All structure figures were prepared using PyMol (http://pymol.sourceforge.net/).

## 5. Experimental section

The reagents (chemicals) were purchased from Lancaster, Acros and Shanghai Chemical Reagent Company, and used without further purification. Analytical thin-layer chromatography (TLC) was performed on HSGF 254 (150–200 μm thickness, Yantai Huiyou Company, China). Yields were not optimized. Melting points were measured in capillary tube on a SGW X-4 melting point apparatus without correction. Microwave experiments under solvent-free conditions were carried out in a domestic microwave oven (Galanz WG800DSL20II-K6). Microwave assisted Suzuki coupling reactions were performed in an InitiatorTM EXP microwave system (Biotage, Inc.) at the specified temperature using the standard mode of operation (the heating process: temperature: 0–250 °C; temperature increase: 2–5 °C/s; pressure range: 0–20 bar; power range: 0–400 W at 2.45 GHz; reaction vials (mL): 0.2–0.5 (EXP), 0.5–2, 2–5, 10–20 (EXP); reaction volumes: 0.2–20 mL). Column chromatography was performed with Combi*Flash*® Companion system (Teledyne Isco, Inc.). Nuclear magnetic resonance (NMR) spectra were performed on a Brucker AMX-400 and AMX-300 NMR (IS as TMS). Chemical shifts were reported in parts per million (ppm, $\delta$) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low- and high-resolution mass spectra (LRMS and HRMS) were given with electric, electrospray and matrix-assisted laser desorption ionization (EI, ESI, and MALDI) produced by Finnigan MAT-95, LCQ-DECA spectrometer and IonSpec 4.7 Tesla. An Agilent 1100 Series HPLC with an Agilent Zorbax Eclipse XDB-$C_{18}$ (4.6 × 50 mm, 5 μm) reversed phase column was used for analytical HPLC analyses. Elemental analyses were performed on an Elementar vario EL I analyzer.

### 5.1. General procedure for the synthesis of compound 6 (P01–P06, P09–P22, and P32)

To a solution of substituted 2-amino-thiophene-3-carboxylic derivatives **5** (0.1 mmol) in 10 mL dry $CH_2Cl_2$ was added an equimolar amount of the appropriate acid anhydrides. The mixture was kept at refluxing overnight under an atmosphere of Argon. The reaction mixture was concentrated under reduced pressure and purified by flash column chromatography. The pure products (**P01–P06**, **P09–P22**, and **P32**) were recrystallized from $CH_2Cl_2$/petroleum ether (PE).

#### 5.1.1. 2-[(3-Carboxy-bicyclo[2.2.1]heptane-2-carbonyl)-amino]-4-naphthalen-2-yl-thiophene-3-carboxylic acid isopropyl ester (P01)

The product was purified by flash column chromatography with ethyl acetate (EA)/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/3.5, v/v) to give a white solid. 63% yield; mp 164–165 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.84 (d, $J$ = 6.3 Hz, 6H), 1.54 (m, 4H), 1.77 (m, 1H), 2.11 (m, 1H), 2.64–2.70 (m, 2H), 3.04 (d, $J$ = 11.4 Hz, 1H), 3.28 (d, $J$ = 11.7 Hz, 1H), 4.96 (m, 1H), 6.64 (s, 1H), 7.39 (d, $J$ = 8.4 Hz, 1H), 7.48 (m, 2H), 7.73–7.86 (m, 4H), 11.5 (s, 1H); LRMS (EI) $m/z$ 477 ($M^+$), 251 (100%); HRMS (EI) $m/z$ calcd for $C_{27}H_{27}NO_5S$ ($M^+$) 477.1610, found 477.1609.

#### 5.1.2. 2-[(3-Carboxy-bicyclo[2.2.1]hept-5-ene-2-carbonyl)-amino]-4-naphthalen-2-yl-thiophene-3-carboxylic acid isopropyl ester (P02)

The product was purified by flash column chromatography with EA/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/3.5, v/v) to give a yellow solid. 75% yield; mp 148–150 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.85 (d, $J$ = 6.3 Hz, 6H), 1.43 (d, $J$ = 8.1 Hz, 1H), 1.57 (d, $J$ = 8.4 Hz, 1H), 2.28 (m, 2H), 3.43 (d, $J$ = 10.2 Hz, 1H), 3.52 (d, $J$ = 10.2 Hz, 1H), 4.97 (m, 1H), 6.34 (m, 1H), 6.47 (m, 1H), 6.63 (s, 1H), 7.39 (d, $J$ = 8.4 Hz, 1H), 7.48 (m, 2H), 7.73–7.86 (m, 4H), 11.5 (s, 1H); LRMS (EI) $m/z$ 475 ($M^+$), 251 (100%); HRMS (EI) $m/z$ calcd for $C_{27}H_{25}NO_5S$ ($M^+$) 475.1453, found 475.1457. Anal. ($C_{27}H_{25}NO_5S{\cdot}0.2H_2O$) C, H, N.

#### 5.1.3. 2-[(2-Carboxy-cyclohexanecarbonyl)-amino]-4-naphthalen-2-yl-thiophene-3-carboxylic acid isopropyl ester (P03)

The product was purified by flash column chromatography with EA/PE (1/2.5, v/v) and recrystallized from $CH_2Cl_2$/PE (1/4, v/v) to give a white solid. 57% yield; mp 204–205 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.85 (d, $J$ = 6.3 Hz, 6H), 1.14–2.24 (m, 8H), 2.93–3.14 (m, 2H), 4.98 (m, 1H), 6.66 (s, 1H), 7.40 (d, $J$ = 8.4 Hz, 1H), 7.48 (m, 2H), 7.74–7.87 (m, 4H), 11.7 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.1, 22.9, 23.4, 25.4, 26.5, 41.7, 42.8, 67.9, 111.4, 115.7, 125.8, 126.1, 126.4, 127.0, 127.7, 128.0, 131.9, 132.5, 135.0, 138.9, 149.1, 164.6, 171.6, 174.6; LRMS (EI) $m/z$ 465 ($M^+$), 251 (100%); HRMS (EI) $m/z$ calcd for $C_{26}H_{27}NO_5S$ ($M^+$) 465.1610, found 465.1598. Anal. ($C_{26}H_{27}NO_5S$) C, H, N.

#### 5.1.4. 2-(2-Carboxy-benzoylamino)-4-naphthalen-2-yl-thiophene-3-carboxylic acid isopropyl ester (P04)

The product was purified by flash column chromatography with EA/PE (1/4, v/v) and recrystallized from $CH_2Cl_2$/PE (1/2, v/v) to give a white solid. 62% yield; mp 158–160 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.85 (d, $J$ = 6.0 Hz, 6H), 4.97 (m, 1H), 6.77 (s, 1H), 7.43–7.89 (m, 10H), 8.13 (d, $J$ = 7.5 Hz, 1H), 11.8 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.6, 68.2, 112.8, 116.2, 125.9, 126.1, 126.5, 127.0, 127.4, 127.6, 127.8, 127.9, 129.8, 130.6, 130.7, 132.0, 132.1, 132.5, 134.7, 135.8, 139.1, 148.4, 164.4, 165.7, 167.3; LRMS (ESI) $m/z$ 458 $[M+H]^+$; HRMS (ESI) $m/z$ calcd for $C_{26}H_{21}NO_5NaS$ $[M+Na]^+$ 482.1038, found 482.1045. Anal. ($C_{26}H_{21}NO_5S$) C, H, N.

#### 5.1.5. 2-(3-Carboxy-propionylamino)-4-naphthalen-2-yl-thiophene-3-carboxylic acid isopropyl ester (P05)

The product was purified by flash column chromatography with EA/PE (1/2, v/v) and recrystallized from $CH_2Cl_2$/PE (1/4, v/v) to give a white solid. 48% yield; mp 154–155 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.85 (d, $J$ = 6.0 Hz, 6H), 2.87 (br, 4H), 4.97 (m, 1H), 6.69 (s, 1H), 7.39–7.62 (m, 3H), 7.75–7.87 (m, 4H), 11.8 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.1, 28.6, 30.7, 67.9, 112.0, 115.8, 125.8, 126.1, 126.5, 126.9, 127.4, 127.7, 127.9, 131.9, 132.5, 134.8, 138.8, 148.2, 164.1, 169.7, 173.5; LRMS (EI) $m/z$ 411 ($M^+$), 251 (100%); HRMS (EI) $m/z$ calcd for $C_{22}H_{21}NO_5S$ ($M^+$) 411.1140, found 411.1145. Anal. ($C_{22}H_{21}NO_5S$) C, H, N.

#### 5.1.6. 4-Biphenyl-4-yl-2-((*Z*)-3-carboxy-acryloylamino)-thiophene-3-carboxylic acid isopropyl ester (P06)

The product was purified by flash column chromatography with EA/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/3, v/v) to give a yellow solid. 83% yield; mp 154–155 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.99 (d, $J$ = 6.0 Hz, 6H), 5.03 (m, 1H), 6.54 (m, 2H), 6.88 (s, 1H), 7.34 (m, 3H), 7.48 (t, $J$ = 7.2 Hz, 2H), 7.64 (m, 4H), 12.3 (s, 1H); LRMS (EI) $m/z$ 465 ($M^+$), 251 (100%); HRMS (EI) $m/z$ calcd for $C_{24}H_{21}NO_5S$ ($M^+$) 435.1140, found 435.1148. Anal. ($C_{24}H_{21}NO_5S$) C, H, N.

#### 5.1.7. 2-[(2-Carboxy-cyclohexanecarbonyl)-amino]-4-naphthalen-2-yl-thiophene-3-carboxylic acid ethyl ester (P09)

The product was purified by flash column chromatography with EA/PE (1/2.5, v/v) and recrystallized from $CH_2Cl_2$/PE (1/4, v/v) to give a white solid. 78% yield; mp 195–197 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.81 (t, $J$ = 7.2 Hz, 3H), 1.53–2.23 (m, 8H), 2.93–3.11 (m, 2H), 4.04 (q, $J$ = 7.2 Hz, 2H), 6.66 (s, 1H), 7.39–7.49 (m, 3H), 7.76–7.83 (m, 4H), 11.6 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 13.4, 22.9, 23.4, 25.5, 26.4, 41.7, 42.7, 60.3, 111.0, 115.8, 125.8, 126.0, 126.4, 126.9, 127.4, 127.8, 127.9, 131.9, 132.5, 134.8, 138.9, 149.2, 165.0, 171.6, 174.6; LRMS (EI) $m/z$ 451 ($M^+$), 251 (100%); HRMS (EI) $m/z$ calcd for $C_{25}H_{25}NO_5S$ ($M^+$) 451.1453, found 451.1445. Anal. ($C_{25}H_{25}NO_5S$) C, H, N.

#### 5.1.8. 2-[(2-Carboxy-cyclohexanecarbonyl)-amino]-4-naphthalen-2-yl-thiophene-3-carboxylic acid methyl ester (P10)

The product was purified by flash column chromatography with EA/PE (1/2.5, v/v) and recrystallized from $CH_2Cl_2$/PE (1/4, v/v) to give a white solid. 70% yield; mp 99–101 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.53–2.29 (m, 8H), 2.92–3.14 (m, 2H), 3.56 (s, 3H), 6.67 (s, 1H), 7.39–7.54 (m, 3H), 7.77–7.88 (m, 4H), 11.6 (s, 1H); LRMS (EI) $m/z$ 437 ($M^+$), 251 (100%); HRMS (EI) $m/z$ calcd for $C_{24}H_{23}NO_5S$ ($M^+$) 437.1297, found 437.1303.

#### 5.1.9. 2-[(2-Carboxy-cyclohexanecarbonyl)-amino]-4-naphthalen-2-yl-thiophene-3-carboxylic acid benzyl ester (P11)

The product was purified by flash column chromatography with EA/PE (1/5, v/v) and recrystallized from $CH_2Cl_2$/PE (1/3, v/v) to give a white solid. 72% yield; mp 144–145 °C; $^1$H NMR (300 MHz, $CDCl_3$): 1.53–2.29 (m, 8H), 2.91–2.95 (m, 1H), 3.10 (br, 1H), 5.05 (s, 2H), 6.62–6.68 (m, 3H), 6.84 (t, $J$ = 7.8 Hz, 2H), 7.04 (t, $J$ = 7.8 Hz, 1H), 7.32 (d, $J$ = 7.8 Hz, 1H), 7.47–7.50 (m, 2H), 7.62 (d, $J$ = 7.8 Hz, 1H), 7.70–7.85 (m, 3H), 11.6 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 22.9, 23.4, 25.4, 26.4, 41.7, 42.8, 51.4, 66.0, 110.9, 115.9, 125.8, 125.9, 126.1, 126.8, 127.3, 127.6, 127.7, 127.8, 132.1, 132.7, 134.8, 134.9, 135.0, 138.9, 149.2, 149.4, 164.7, 171.7, 174.6; LRMS (EI) $m/z$ 513 ($M^+$), 91 (100%); HRMS (EI) $m/z$ calcd for $C_{30}H_{27}NO_5S$ ($M^+$) 513.1610, found 513.1620. Anal. ($C_{30}H_{27}NO_5S{\cdot}0.8H_2O$) C, H, N.

#### 5.1.10. 2-[(3-Carboxy-bicyclo[2.2.1]hept-5-ene-2-carbonyl)-amino]-4-naphthalen-2-yl-thiophene-3-carboxylic acid ethyl ester (P12)

The product was purified by flash column chromatography with EA/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/3.5, v/v) to give a light yellow solid. 65% yield; mp 123–124 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.81 (t, $J$ = 7.2 Hz, 3H), 1.43 (d, $J$ = 8.7 Hz, 1H), 1.57 (d, $J$ = 8.7 Hz, 1H), 2.27 (m, 2H), 3.39–3.53 (m, 2H), 4.04 (q, $J$ = 7.2 Hz, 2H), 6.34 (m, 1H), 6.46 (m, 1H), 6.63 (s, 1H), 7.39 (d, $J$ = 8.4 Hz, 1H), 7.48 (m, 2H), 7.74–7.76 (m, 4H), 11.4 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 13.4, 45.7, 46.3, 48.4, 49.1, 49.2, 54.9, 60.1, 110.9, 115.7, 125.8, 126.0, 126.4, 126.8, 127.4, 127.8,

131.9, 132.5, 134.1, 134.9, 135.8, 138.7, 149.0, 164.7, 169.8, 173.0; LRMS (ESI) *m/z* 460 $[M-H]^-$; HRMS (ESI) *m/z* calcd for $C_{26}H_{23}NO_5S$-Na $[M+Na]^+$ 484.1195, found 484.1187. Anal. ($C_{26}H_{23}NO_5S\cdot H_2O$) C, H, N.

#### 5.1.11. 2-[(3-Carboxy-bicyclo[2.2.1]hept-5-ene-2-carbonyl)-amino]-4-phenyl-thiophene-3-carboxylic acid ethyl ester (P13)

The product was purified by flash column chromatography with EA/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/3, v/v) to give a yellow solid. 71% yield; mp 162–163 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.92 (t, *J* = 7.2 Hz, 3H), 1.42 (d, *J* = 7.8 Hz, 1H), 1.57 (d, *J* = 8.1 Hz, 1H), 2.26 (m, 2H), 3.41 (d, *J* = 10.2 Hz, 1H), 3.50 (d, *J* = 9.9 Hz, 1H), 4.06 (q, *J* = 7.2 Hz, 2H), 6.33 (m, 1H), 6.46 (m, 1H), 6.55 (s, 1H), 7.25–7.33 (m, 5H), 11.4 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 13.3, 45.7, 46.2, 48.4, 49.0, 49.1, 60.1, 110.9, 115.2, 126.8, 127.4, 128.8, 134.1, 135.7, 137.3, 138.8, 148.7, 164.7, 169.7, 173.0; LRMS (ESI) *m/z* 411 ($M^+$), 201 (100%); HRMS (EI) *m/z* calcd for $C_{22}H_{21}NO_5S$ ($M^+$) 411.1140, found 411.1143. Anal. ($C_{22}H_{21}NO_5S$) C, H, N.

#### 5.1.12. 2-[(2-Carboxy-cyclohexanecarbonyl)-amino]-4-(4-nitro-phenyl)-thiophene-3-carboxylic acid isopropyl ester (P14)

The product was purified by flash column chromatography with EA/PE (1/2, v/v) and recrystallized from $CH_2Cl_2$/PE (1/1, v/v) to give a yellow solid. 73% yield; mp 195–196 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.97 (d, *J* = 6.3 Hz, 6H), 1.48–2.23 (m, 8H), 2.93 (m, 1H), 3.12 (m, 1H), 5.03 (m, 1H), 6.64 (s, 1H), 7.45 (d, *J* = 6.9 Hz, 1H), 8.21 (d, *J* = 6.9 Hz, 1H), 11.6 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.1, 22.8, 23.4, 25.3, 26.4, 41.7, 42.7, 68.4, 110.8, 116.9, 122.6, 130.4, 136.8, 144.4, 146.4, 149.6, 164.1, 171.7, 174.6; LRMS (EI) *m/z* 460 ($M^+$), 246 (100%); HRMS (EI) *m/z* calcd for $C_{22}H_{24}N_2O_7S$ ($M^+$) 460.1304, found 460.1293. Anal. ($C_{22}H_{24}N_2O_7S$) C, H, N.

#### 5.1.13. 2-[(3-Carboxy-bicyclo[2.2.1]hept-5-ene-2-carbonyl)-amino]-4-(4-fluoro-phenyl)-thiophene-3-carboxylic acid ethyl ester (P15)

The product was purified by flash column chromatography with EA/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/2, v/v) to give a yellow solid. 73% yield; mp 164–165 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.96 (t, *J* = 7.2 Hz, 3H), 1.43 (m, 1H), 1.56 (m, 1H), 2.26 (m, 2H), 3.38–3.52 (m, 2H), 4.08 (q, *J* = = 7.2 Hz, 2H), 6.32 (m, 1H), 6.44 (m, 1H), 6.52 (s, 1H), 7.01 (m, 2H), 7.23 (m, 2H), 11.4 (s, 1H); LRMS (EI) *m/z* 429 ($M^+$), 219 (100%); HRMS (EI) *m/z* calcd for $C_{22}H_{20}FNO_5S$ ($M^+$) 429.1046, found 429.1041.

#### 5.1.14. 2-(2-Carboxy-benzoylamino)-4-phenyl-thiophene-3-carboxylic acid isopropyl ester (P16)

The product was purified by flash column chromatography with EA/PE (1/4, v/v) and recrystallized from $CH_2Cl_2$/PE (1/2, v/v) to give a white solid. 68% yield; mp 190–191 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.93 (d, *J* = 6.0 Hz, 6H), 4.95 (m, 1H), 6.66 (s, 1H), 7.25–7.35 (m, 5H), 7.58–7.69 (m, 3H), 8.09 (d, *J* = 7.8 Hz, 1H), 11.8 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.1, 68.1, 112.8, 115.7, 126.9, 127.4, 127.5, 128.9, 129.8, 130.6, 130.8, 132.1, 135.8, 137.1, 139.2, 148.1, 164.4, 165.7, 167.3; LRMS (EI) *m/z* 409 ($M^+$), 201 (100%); HRMS (EI) *m/z* calcd for $C_{22}H_{19}NO_5S$ ($M^+$) 409.0984, found 409.0984. Anal. ($C_{22}H_{19}NO_5S$) C, H, N.

#### 5.1.15. 4-(4-Bromo-phenyl)-2-(2-carboxy-benzoylamino)-thiophene-3-carboxylic acid ethyl ester (P17)

The product was purified by flash column chromatography with EA/PE (1/4, v/v) and recrystallized from $CH_2Cl_2$/PE (1/2, v/v) to give a light green solid. 65% yield; mp 161–163 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.96 (t, *J* = 7.2 Hz, 3H), 4.04 (q, *J* = 7.2 Hz, 2H), 6.64 (s, 1H), 7.20 (d, *J* = 8.7 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 3H), 7.61–7.69 (m, 4H), 8.09 (d, *J* = 7.8 Hz, 1H), 11.7 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 13.3, 60.5, 112.3, 116.3, 120.3, 127.6. 129.8, 130.4, 130.7, 130.8, 131.0, 132.0, 135.7, 136.2, 137.8, 148.3, 164.5, 165.8, 167.3; LRMS (EI) *m/z* 475 ($M^+$), 281 (100%); HRMS (EI) *m/z* calcd for $C_{21}H_{16}BrNO_5S$ ($M^+$) 472.9933, found 472.9946. Anal. ($C_{21}H_{16}BrNO_5S$) C, H, N.

#### 5.1.16. 2-[(3-Carboxy-bicyclo[2.2.1]hept-5-ene-2-carbonyl)-amino]-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid isopropyl ester (P18)

The product was purified by flash column chromatography with EA/PE (1/2, v/v) and recrystallized from $CH_2Cl_2$/PE (1/4, v/v) to give a white solid. 81% yield; mp 181–182 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.37 (d, *J* = 6.3 Hz, 6H), 1.39 (m, 1H), 1.52 (m, 1H), 1.77 (m, 2H), 2.26 (m, 2H), 2.60 (m, 2H), 2.74 (m, 2H), 3.23 (m, 2H), 3.33–3.47 (m, 2H), 5.20 (m, 1H), 6.27 (m, 1H), 6.41 (m, 1H), 11.5 (s, 1H); LRMS (EI) *m/z* 403 ($M^+$), 179 (100%); HRMS (EI) *m/z* calcd for $C_{21}H_{25}NO_5S$ ($M^+$) 403.1453, found 403.1437.

#### 5.1.17. 4-Biphenyl-4-yl-2-[(3-carboxy-bicyclo[2.2.1]hept-5-ene-2-carbonyl)-amino]-thiophene-3-carboxylic acid isopropyl ester (P19)

The product was purified by flash column chromatography with EA/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/2, v/v) to give a yellow solid. 53% yield; mp 172–173 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.98 (d, *J* = 6.0 Hz, 6H), 1.43 (d, *J* = 8.1 Hz, 1H), 1.57 (d, *J* = 8.1 Hz, 1H), 2.27 (m, 2H), 3.42 (d, *J* = 10.2 Hz, 1H), 3.51 (d, *J* = 10.2 Hz, 1H), 5.00 (m, 1H), 6.34 (m, 1H), 6.46 (m, 1H), 6.59 (s, 1H), 7.35 (m, 3H), 7.46 (t, *J* = 7.2 Hz, 2H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 7.2 Hz, 2H), 11.5 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.1, 45.7, 46.3, 48.4, 49.1, 49.2, 67.9, 111.2, 115.1, 125.6, 126.5, 127.4, 128.9, 129.5, 134.1, 135.7, 136.6, 138.5, 138.7, 139.9, 148.8, 164.3, 169.7, 173.0; LRMS (ESI) *m/z* 500 $[M-H]^-$; HRMS (ESI) *m/z* calcd for $C_{29}H_{27}NO_5NaS$ $[M+Na]^+$ 524.1508, found 524.1504. Anal. ($C_{29}H_{27}NO_5S$) C, H, N.

#### 5.1.18. 4-Biphenyl-4-yl-2-[(2-carboxy-cyclohexanecarbonyl)-amino]-thiophene-3-carboxylic acid isopropyl ester (P20)

The product was purified by flash column chromatography with EA/PE (1/2, v/v) and recrystallized from $CH_2Cl_2$/PE (1/3, v/v) to give a yellow solid. 61% yield; mp 242–243 °C; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 0.98 (d, *J* = 6.3 Hz, 6H), 1.30–2.10 (m, 8H), 2.93 (m, 1H), 3.38 (m, 1H), 4.96 (m, 1H), 6.92 (s, 1H), 7.31–7.80 (m, 9H), 11.3 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.1, 22.9, 23.4, 25.4, 26.4, 41.7, 42.7, 54.9, 68.1, 111.4, 115.2, 125.6, 127.4, 128.9, 129.5, 136.5, 138.6, 138.7, 139.9, 149.0, 164.5, 171.5, 174.6; LRMS (EI) *m/z* 491 ($M^+$), 277 (100%); HRMS (EI) *m/z* calcd for $C_{28}H_{29}NO_5S$ ($M^+$) 491.1766, found 491.1772. Anal. ($C_{28}H_{29}NO_5S\cdot 0.7H_2O$) C, H, N.

#### 5.1.19. 4-Biphenyl-4-yl-2-(2-carboxy-benzoylamino)-thiophene-3-carboxylic acid isopropyl ester (P21)

The product was purified by flash column chromatography with EA/PE (1/4, v/v) and recrystallized from $CH_2Cl_2$/PE (1/2, v/v) to give a white solid. 52% yield; mp 161–162 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.96 (d, *J* = 6.0 Hz, 6H), 4.97 (m, 1H), 6.71 (s, 1H), 7.35 (m, 3H), 7.46 (t, *J* = 7.2 Hz, 2H), 7.56–7.68 (m, 7H), 8.09 (d, *J* = 7.8 Hz, 1H), 11.8 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.1, 68.3, 112.8, 115.8, 125.7, 126.5, 127.4, 127.6, 129.0, 129.5, 129.9, 130.6, 130.8, 132.1, 135.8, 136.2, 138.8, 138.9, 139.9; LRMS (EI) *m/z* 485 ($M^+$), 277 (100%); HRMS (ESI) *m/z* calcd for $C_{28}H_{23}NO_5S$ ($M^+$) 485.1297, found 485.1299. Anal. ($C_{28}H_{23}NO_5S$) C, H, N.

#### 5.1.20. 2-[(3-Carboxy-bicyclo[2.2.1]hept-5-ene-2-carbonyl)-amino]-4-(4′-chloro-biphenyl-4-yl)-thiophene-3-carboxylic acid isopropyl ester (P22)

The product was purified by flash column chromatography with EA/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/3, v/v) to give

a yellow solid. 75% yield; mp 192–194 °C; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 0.98 (d, $J$ = 6.3 Hz, 6H), 1.42–1.59 (dd, $J$ = 8.4, 12.3 Hz, 2H), 2.28 (m, 2H), 3.41–3.53 (m, 2H), 5.01 (m, 1H), 6.32 (m, 1H), 6.44 (m, 1H), 6.58 (s, 1H), 7.33 (d, $J$ = 7.8 Hz, 2H), 7.40–7.57 (m, 6H), 11.49 (s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 21.4, 46.1, 47.3, 49.0, 49.1, 50.3, 68.4, 111.7, 115.1, 125.7, 128.2, 128.9, 129.8, 133.3, 133.8, 136.3, 137.5, 138.4, 139.1, 139.3, 149.9, 165.6, 169.3, 176.2; LRMS (EI) $m/z$ 535 ($M^+$), 311 (100%); HRMS (EI) $m/z$ calcd for $C_{29}H_{26}ClNO_5S$ ($M^+$) 535.1220, found 535.1229.

#### 5.1.21. 2-[(3-Carboxy-bicyclo[2.2.1]hept-5-ene-2-carbonyl)-amino]-5-phenyl-thiophene-3-carboxylic acid isopropyl ester (P32)

The product was purified by flash column chromatography with EA/PE (1/2, v/v) and recrystallized from $CH_2Cl_2$/PE (1/4, v/v) to give a yellow solid. 93% yield; mp 192–194 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.36 (d, $J$ = 6.3 Hz, 6H), 1.42 (m, 1H), 1.57 (m, 1H), 2.26 (m, 2H), 3.38–3.52 (m, 2H), 5.23 (m, 1H), 6.32 (m, 1H), 6.45 (m, 1H), 7.25–7.37 (m, 2H), 7.57 (d, $J$ = 8.4 Hz, 2H), 11.2 (s, 1H); LRMS (ESI) $m/z$ 424 [M−H]$^-$; HRMS (ESI) $m/z$ calcd for $C_{23}H_{23}NO_5NaS$ [M+Na]$^+$ 448.1195, found 448.1175.

### 5.2. 2-Benzoylamino-4-biphenyl-4-yl-thiophene-3-carboxylic acid isopropyl ester (P07)

To a solution of 2-amino-4-biphenyl-4-yl-thiophene-3-carboxylic acid isopropyl ester (0.1 mmol) in dry $CH_2Cl_2$ was added with 0.105 mmol of benzoyl chloride and 0.15 mmol of triethylamine (TEA). The mixture was stirred at room temperature for 2–3 h until the starting material disappeared at TLC analysis (EA/PE = 1/8). The reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography to give a white solid. Mp 131–133 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.02 (d, $J$ = 6.3 Hz, 6H), 5.08 (m, 1H), 6.71 (s, 1H), 7.35 (m, 3H), 7.46 (t, $J$ = 7.2 Hz, 2H), 7.56–7.68 (m, 7H), 8.09 (m, 2H), 12.4 (s, 1H); LRMS (ESI) $m/z$ 442 [M+H]$^+$; HRMS (ESI) $m/z$ calcd for $C_{27}H_{23}NO_5SNa$ [M+Na]$^+$ 464.1296, found 464.1232.

### 5.3. 2-(2-Methoxycarbonyl-benzoylamino)-4-biphenyl-4-yl-thiophene-3-carboxylic acid isopropyl ester (P08)

To a solution of **P21** (0.1 mmol) in methanol (10 mL) was added 300 μL concentrated sulfuric acid, and the mixture was kept stirring under reflux for 2–3 h. The resulting solution was concentrated under vacuum, and the residue was dissolved in EA (30 mL), washed with saturated $NaHCO_3$ solution, brine, dried with $Na_2SO_4$ and concentrated. The product was purified by flash column chromatography with EA/PE (1/10, v/v) and recrystallized from $CH_2Cl_2$/PE (1/2, v/v) to give a light pink solid. 90% yield; mp 178–180 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.98 (d, $J$ = 6.0 Hz, 6H), 3.90 (s, 3H), 4.97 (m, 1H), 6.72 (s, 1H), 7.35 (m, 2H), 7.48 (t, $J$ = 7.5 Hz, 2H), 7.56–7.68 (m, 7H), 7.98 (d, $J$ = 7.8 Hz, 1H), 11.7 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.1, 52.6, 68.3, 113.3, 116.0, 125.8, 126.5, 127.4, 127.7, 128.9, 129.5, 129.7, 129.8, 131.1, 132.4, 135.4, 136.1, 138.8, 139.9, 147.8, 164.3, 165.1, 166.6; LRMS (ESI) $m/z$ 500 [M+H]$^+$; HRMS (ESI) $m/z$ calcd for $C_{29}H_{25}NO_5SNa$ [M+Na]$^+$ 522.1351, found 522.1360.

### 5.4. General procedure for the synthesis of compounds P23–P31

A mixture of 4-(4-bromo-phenyl)-2-(2-carboxy-benzoylamino)-thiophene-3-carboxylic acid ethyl ester (**P17**) (0.1 mmol), boronic acid derivatives (0.12 mmol), $Pd(PPh_3)_4$ (0.01 mmol), tetraethylammonium bromide (TBAB: 0.1 mmol), and $Na_2CO_3$ (0.2 mmol) was stirred in water (3–5 mL) under an atmosphere of argon. The vial was sealed and the mixture was then irradiated for 10 min at 150 °C. After the reaction was cooled to ambient temperature, the crude reaction mixture was extracted three times with EA (15 mL × 3). The combined organic phase was washed with brine, dried with $Na_2SO_4$, filtered, and concentrated. The residue was purified by column chromatography on Combiflash, and recrystallized to provide the desired products.

#### 5.4.1. 2-(2-Carboxy-benzoylamino)-4-(2′-methoxy-biphenyl-4-yl)-thiophene-3-carboxylic acid ethyl ester (P23)

The product was purified by flash column chromatography with EA/PE (1/4, v/v) and recrystallized from $CH_2Cl_2$/PE (1/1, v/v) to give a white solid. 85% yield; mp 192–194 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.96 (t, $J$ = 7.2 Hz, 3H), 3.83 (s, 3H), 4.09 (q, $J$ = 6.6 Hz, 2H), 6.75 (s, 1H), 7.00–7.07 (m, 2H), 7.35 (m, 4H), 7.51 (d, $J$ = 8.1 Hz, 2H), 7.62–7.70 (m, 3H), 8.12 (d, $J$ = 7.5 Hz, 1H), 11.8 (s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 13.2, 55.5, 60.6, 111.2, 112.6, 115.7, 120.8, 127.7, 128.5, 128.6, 128.8, 129.3, 130.5, 130.7, 131.0, 131.6, 132.8, 135.8, 136.1, 137.3, 139.9, 149.6, 156.5. 166.1, 166.2, 168.5; LRMS (EI) $m/z$ 501 ($M^+$), 307 (100%); HRMS (ESI) $m/z$ calcd for $C_{28}H_{23}NO_6S$ ($M^+$) 501.1246, found 501.1252. Anal. ($C_{28}H_{23}NO_6S{\cdot}0.4H_2O$) C, H, N.

#### 5.4.2. 2-(2-Carboxy-benzoylamino)-4-(3′-methoxy-biphenyl-4-yl)-thiophene-3-carboxylic acid ethyl ester (P24)

The product was purified by flash column chromatography with EA/PE (1/4, v/v) and recrystallized from $CH_2Cl_2$/PE (1/1, v/v) to give a white solid. 86% yield; mp 210–211 °C; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 0.94 (t, $J$ = 7.5 Hz, 3H), 3.83 (s, 3H), 4.09 (q, $J$ = 7.5 Hz, 2H), 6.94–6.98 (dd, $J$ = 1.2, 7.2 Hz, 1H), 7.07 (s, 1H), 7.21–7.29 (m, 2H), 7.38–7.43 (m, 3H), 7.62–7.75 (m, 5H), 7.95 (d, $J$ = 7.5 Hz, 1H), 11.4 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 13.4, 55.1, 60.5, 112.0, 112.7, 113.0, 116.0, 118.9, 125.9, 127.6, 129.4, 129.8, 130.0, 130.7, 130.8, 132.1, 135.9, 136.2, 138.7, 141.4, 148.0, 159.8, 164.7, 165.8, 167.3; LRMS (EI) $m/z$ 501 ($M^+$), 307 (100%); HRMS (ESI) $m/z$ calcd for $C_{28}H_{23}NO_6S$ ($M^+$) 501.1246, found 501.1241.

#### 5.4.3. 2-(2-Carboxy-benzoylamino)-4-(4′-methoxy-biphenyl-4-yl)-thiophene-3-carboxylic acid ethyl ester (P25)

The product was purified by flash column chromatography with EA/PE (1/4, v/v) and recrystallized from $CH_2Cl_2$/PE (1/1, v/v) to give a white solid. 82% yield; mp 180–181 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.94 (t, $J$ = 6.9 Hz, 3H), 3.87 (s, 3H), 4.09 (q, $J$ = 6.6 Hz, 2H), 6.75 (s, 1H), 7.02 (m, 2H), 7.39 (m, 2H), 7.53–7.70 (m, 7H), 8.12 (d, $J$ = 7.5 Hz, 1H), 11.8 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 13.4, 55.2, 60.5, 112.8, 114.4, 115.9, 125.3, 127.6, 129.3, 129.8, 130.7, 132.0, 132.2, 135.3, 135.8, 138.5, 138.8, 147.9, 158.9, 164.8, 165.8, 167.3; LRMS (EI) $m/z$ 501 ($M^+$), 307 (100%); HRMS (ESI) $m/z$ calcd for $C_{28}H_{23}NO_6S$ ($M^+$) 501.1246, found 501.1239. Anal. ($C_{28}H_{23}NO_6S{\cdot}0.3H_2O$) C, H, N.

#### 5.4.4. 2-(2-Carboxy-benzoylamino)-4-(4′-fluoro-biphenyl-4-yl)-thiophene-3-carboxylic acid ethyl ester (P26)

The product was purified by flash column chromatography with EA/PE (1/3.5, v/v) and recrystallized from $CH_2Cl_2$/PE (1/1, v/v) to give a white solid. 82% yield; mp 179–180 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.94 (t, $J$ = 7.2 Hz, 3H), 4.07 (q, $J$ = 7.2 Hz, 2H), 6.73 (s, 1H), 7.14 (t, $J$ = 8.4 Hz, 2H), 7.38 (d, $J$ = 8.1 Hz, 2H), 7.51–7.70 (m, 8H), 8.12 (d, $J$ = 7.5 Hz, 1H), 11.8 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 13.4, 60.5, 112.7, 115.7, 115.9, 116.0, 125.7, 127.6, 128.5, 128.6, 129.4, 129.8, 130.6, 130.8, 132.1, 135.7, 136.0, 136.3, 137.8, 138.7, 148.0, 160.1, 163.1, 164.7, 165.7, 167.3; LRMS (EI) $m/z$ 489 ($M^+$), 295 (100%); HRMS (ESI) $m/z$ calcd for $C_{27}H_{20}FNO_5S$ ($M^+$) 489.1046, found 489.1064. Anal. ($C_{27}H_{20}FNO_5S$) C, H, N.

#### 5.4.5. 2-(2-Carboxy-benzoylamino)-4-(4′-trifluoromethyl-biphenyl-4-yl)-thiophene-3-carboxylic acid ethyl ester (P27)

The product was purified by flash column chromatography with EA/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/1, v/v) to give a white solid. 83% yield; mp 200–202 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.94 (t, $J$ = 7.2 Hz, 3H), 4.07 (q, $J$ = 7.2 Hz, 2H), 6.74 (s, 1H), 7.42 (d, $J$ = 8.1 Hz, 2H), 7.68–7.72 (m, 10H), 8.12 (d, $J$ = 6.9 Hz, 1H), 11.8 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 13.4, 60.7, 112.3, 115.9, 125.7, 126.2, 127.2, 127.7, 128.9, 129.9, 130.9, 137.6, 132.9, 136.0, 137.7, 138.4, 139.2, 144.3, 149.9, 166.0, 166.1, 169.3; LRMS (EI) $m/z$ 539 ($M^+$), 345 (100%); HRMS (ESI) $m/z$ calcd for $C_{28}H_{20}F_3NO_5S$ ($M^+$) 539.1014, found 539.1041. Anal. ($C_{28}H_{20}F_3NO_5S$) C, H, N.

#### 5.4.6. 2-(2-Carboxy-benzoylamino)-4-(3′-chloro-4′-fluoro-biphenyl-4-yl)-thiophene-3-carboxylic acid ethyl ester (P28)

The product was purified by flash column chromatography with EA/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/1, v/v) to give a white solid. 84% yield; mp 159–160 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.94 (t, $J$ = 6.9 Hz, 3H), 4.07 (q, $J$ = 6.6 Hz, 2H), 6.71 (s, 1H), 7.19–7.69 (m, 10H), 8.10 (d, $J$ = 7.5 Hz, 1H), 11.7 (s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 13.4, 60.5, 112.6, 116.1, 117.2, 117.4, 120.0, 120.2, 125.9, 127.1, 127.2, 127.5, 128.4, 129.5, 129.8, 130.6, 130.8, 132.1, 135.8, 136.4, 136.6, 137.7, 138.4, 148.0, 155.6, 158.0, 164.7, 165.8, 167.3; LRMS (EI) $m/z$ 523 ($M^+$), 505 (100%); HRMS (ESI) $m/z$ calcd for $C_{27}H_{19}ClFNO_5S$ ($M^+$) 523.0656, found 523.0678. Anal. ($C_{27}H_{19}ClFNO_5S$) C, H, N.

#### 5.4.7. 4-Biphenyl-3-yl-2-(2-carboxy-benzoylamino)-thiophene-3-carboxylic acid isopropyl ester (P29)

The product was purified by flash column chromatography with EA/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/1, v/v) to give a white solid. 84% yield; mp 131–133 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.87 (d, $J$ = 6.3 Hz, 6H), 4.93 (m, 1H), 6.72 (s, 1H), 7.26–7.69 (m, 12H), 8.10 (d, $J$ = 7.2 Hz, 1H), 11.8 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.0, 68.1, 112.9, 115.9, 125.3, 126.7, 127.3, 127.5, 127.6, 128.0, 128.2, 129.0, 129.8, 130.6, 130.8, 132.1, 135.8, 136.4, 137.7, 139.1, 139.4, 140.0, 148.2, 164.4, 165.8, 167.3; LRMS (EI) $m/z$ 485 ($M^+$), 277 (100%); HRMS (ESI) $m/z$ calcd for $C_{28}H_{23}NO_5S$ ($M^+$) 485.1297, found 485.1249. Anal. ($C_{28}H_{23}NO_5S{\cdot}0.2H_2O$) C, H, N.

#### 5.4.8. 4-Biphenyl-2-yl-2-(2-carboxy-benzoylamino)-thiophene-3-carboxylic acid ethyl ester (P30)

The product was purified by flash column chromatography with EA/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/1, v/v) to give a white solid. 80% yield; mp 99–101 °C; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 0.78 (t, $J$ = 6.9 Hz, 3H), 3.64 (q, $J$ = 6.9 Hz, 2H), 6.89 (s, 1H), 7.12–7.45 (m, 9H), 7.61–7.72 (m, 3H), 7.82 (d, $J$ = 7.2 Hz, 1H), 11.2 (s, 1H); LRMS (EI) $m/z$ 471 ($M^+$); HRMS (ESI) $m/z$ calcd for $C_{27}H_{21}NO_5S$ ($M^+$) 471.1140, found 471.1160.

#### 5.4.9. 4-Biphenyl-4-yl-2-(2-carboxy-benzoylamino)-5-methyl-thiophene-3-carboxylic acid ethyl ester (P31)

The product was purified by flash column chromatography with EA/PE (1/3, v/v) and recrystallized from $CH_2Cl_2$/PE (1/1, v/v) to give a white solid. 82% yield; mp 200–201 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.80 (d, $J$ = 6.9 Hz, 3H), 2.28 (s, 3H), 3.96 (q, $J$ = 6.9 Hz, 2H), 6.72 (s, 1H), 7.26–7.69 (m, 12H), 8.12 (d, $J$ = 6.0 Hz, 1H), 11.6 (s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 13.0, 13.2, 60.4, 113.4, 126.2, 126.9, 127.2, 127.7, 128.8, 129.2, 130.2, 130.9, 131.6, 132.9, 134.7, 135.9, 136.3, 139.6, 139.4, 141.0, 146.4, 164.4, 165.8, 166.1, 169.7; LRMS (EI) $m/z$ 485 ($M^+$), 277 (100%); HRMS (ESI) $m/z$ calcd for $C_{28}H_{23}NO_5S$ ($M^+$) 485.1297, found 485.1292.

### 5.5. Biological assay

#### 5.5.1. Construction, expression, and purification of proteins

The vector pGEX4T-1-TC-PTP$_{1-288}$ and pGEX4T-1-PTP1B$_{1-321}$ were constructed with *BamH*I–*Hind*III and *BamH*I–*Eco*RI sites, respectively, using a PTP1B or TC45 vector as templates which were kindly provided by Professor Nicholas K. Tonks (Cold Spring Harbor Laboratory, NY, USA). Vectors pGEX2T-LAR$_{1282-1888}$ and pGEX2T-CD45$_{560-1256}$ were kindly donated by Professor Rafael Pulido (Centro de Investigación Príncipe Felipe, Valencia 46013, Spain). The plasmid pGEX2T-PTP$\alpha_{182-803}$ was kindly provided by Professor Frank R. Jirik (Department of Biochemistry and Molecular Biology, University of Calgary, Canada). Plasmids were transformed into *E. coli* BL21 (DE3) separately and purification of the fusion proteins was carried out following our previously reported method.[12] Briefly, overnight cultures were diluted 1:100 into 1 L LB medium and grew at 37 °C until the absorbance at 600 nm reached 0.6. The recombinant proteins were induced by isopropyl β-D-thiogalactoside (IPTG) (0.2 mM) for another 6 h and cells were harvested by centrifuging for 10 min at 4 °C. Then the cells were frozen at −80 °C for storage. GST-fused proteins were thus purified according to manufacturer's instructions (Amersham Pharmacia).

#### 5.5.2. Enzyme activity assay

The enzymatic system of PTP1B contained 5 mM *p*-nitrophenyl phosphate (*p*NPP), 0.03 μM GST-PTP1B$_{1-321}$, and reaction buffer (20 mM HEPES, 150 mM NaCl, and 1 mM EDTA, pH 7.0). After incubation of compounds for 10 min, reactions were initiated by adding *p*NPP. The amount of produced *p*NP was measured by detecting absorbance at 405 nm using microplate spectrophotometer (Bio-Rad). $IC_{50}$ value was calculated by fitting data with Origin software. To determine inhibition type, DMSO, or compounds were incubated with different concentration of *p*NPP (1 mM–5 mM), respectively, and initial velocities were measured. The inhibition type was determined using the double-reciprocal plots. In experiments to determine the selectivity over PTPases, the enzymatic systems for other PTPases were the same as it for PTP1B.

#### 5.5.3. AKT activation assay

CHO-K1 cells were cultured in F-12 Ham medium supplemented with 10% FBS, penicillin–streptomycin (50 U/mL) at 37 °C in a humidified atmosphere with 5% $CO_2$. Cells were starved and incubated subsequently with **P28** (10 μM) or **3** (10 μM) for 2 h. AKT phosphorylation and total AKT protein levels were immunoblotted with anti-phospho-AKT (Ser473) and anti-AKT primary antibodies. The loading volumes were normalized by anti-GAPDH.

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## Supplementary data

Supplementary data (Figs. S1 and S2, Table S1 of element analysis, and Table S2 of HPLC purity) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.01.055.

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