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Monocyclic thiophenes as protein tyrosine phosphatase 1B inhibitors: Capturing interactions with Asp48

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Abstract—A series of monocyclic thiophenes was designed and synthesized as PTP1B inhibitors. Guided by X-ray co-crystal structural information and computational modeling, rational design led to key interactions with Asp48 and improved inhibitory potency against PTP1B.

Protein tyrosine phosphatase (PTP1B) is an intracellular protein tyrosine phosphatase expressed in insulin responsive tissues[1] and shown to be a negative regulator of insulin signaling as evidenced by studies with PTP1B knockout mice[2] as well as studies using anti-sense oligonucleotides.[3] It is thus considered as one of the most promising therapeutic targets[2,3] among the large PTPase family for potential treatment of type 2 diabetes and obesity. Vast efforts toward the development of PTP1B inhibitors have been disclosed.[4,5] We have recently reported ring-fused thiophenes as PTP1B inhibitors (Fig. 1).[6] A more potent and efficient monocyclic thiophene series is reported herein.

While the fused thiophenes were shown to be reversible and competitive PTP1B inhibitors, close examination of X-ray co-crystal structures suggested that the second and the third fused ring might not be necessary for binding to the enzyme active site. Indeed, removal of the fused rings from the parent compound **1** (R = H, K_i = 230 μM) afforded the monocyclic thiophene **2** with comparable activity (K_i = 160 μM, Fig. 2). Compound **2** was also shown to be a reversible and competitive PTP1B inhibitor.

The X-ray co-crystal structure of PTP1B and **2** is shown in Figure 3. In this structure, the WPD-loop is in the catalytically competent closed conformation and the aromatic thiophene ring is π-stacking between Phe182 and Tyr46, while the C2 and C3 carboxylates provide opportunities for a salt bridge formation with Lys120 and Arg221, respectively. It was also observed that the ether oxygen at the C3 position forms a network of water-mediated hydrogen bonds with Ala217, Arg221, and other residues in the active site. More interestingly, compared to the fused thiophene systems, the C4 bromo group more tightly packs against Ile219, likely contributing to the better inhibition against PTP1B. Replacement of this group with smaller or larger substituents led to diminished inhibitory activity against PTP1B (Scheme 1 and Table 1) as predicted by modeling. Smaller groups such as H or Me (Table 1) have reduced van der Waals and hydrophobic interaction with Ile219, while the larger groups are not sterically accommodated as revealed by the X-ray co-crystal structure and subsequent molecular modeling.

In contrast, introduction of the C5-substitution turned out to be much more productive. For instance, incorporation of an additional bromo group on the C5 position gave compound **10** (K_i = 18 μM, Table 2) synthesized according to the procedures in Scheme 1, displaying a 9-fold increase in inhibition against PTP1B in comparison with compound **2** (K_i = 160 μM, Table 1). Replacing the bromo group with a phenyl ring in compound **11**

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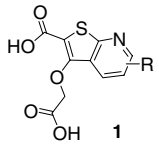


Figure 1. Bicyclic thiophenes as PTP1B inhibitors.

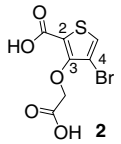


Figure 2. Reversible and competitive monocyclic thiophene **2** as PTP1B inhibitor.

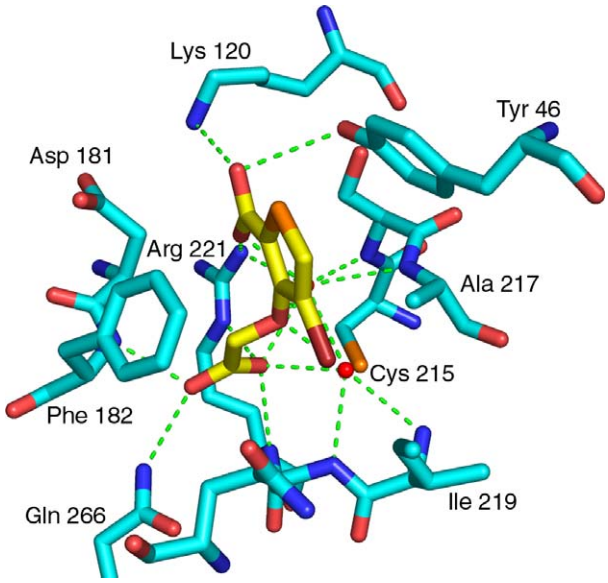
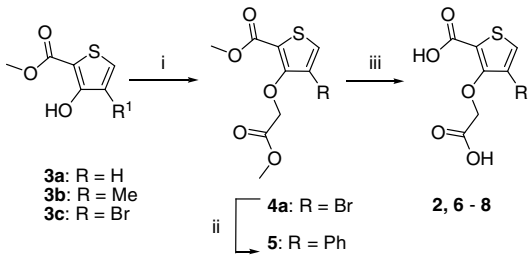


Figure 3. X-ray co-crystal structure of **2** and PTP1B (PBD code: 2HB1).

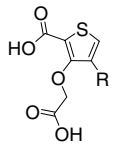


Scheme 1. Reagents: (i) $BrCH_2CO_2Me$, K_2CO_3, DMF (99%); (ii) $Pd(PPh_3)_4$, $PhB(OH)_2$, KF, THF/H_2O (78%); (iii) LiOH, THF/H_2O (90–99%).

(K_i = 3.2 μM, Table 2) led to another 5- to 6-fold increase in potency.

A panel of aryl motifs was then examined. It was shown that a variety of aromatic groups could be accommodated at the C5-position (Scheme 2 and Table 2). It was also noticed that selective Suzuki coupling could be accomplished with non-aqueous solvent such as THF.

Table 1. The effect of C4-substitution on the inhibition against PTP1B.



Compound	R	K_i[a] (μM)
2	Br	160
6	H	>1000
7	Me	>1000
8	Ph	>1000

[a] Enzymatic assay[6] was carried out at room temperature in 96-well plates. To an assay buffer, containing 50 mM of 3,4-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), were added pNPP then compounds (at eight different concentrations). The reaction was initiated by addition of PTP1B (1–299) at a final concentration of 10 or 100 nM. The initial rate of PTPase-catalyzed hydrolysis of 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.

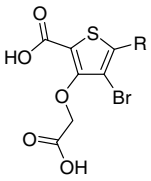
C4,5-Di-aryl thiophene, that is, **16** (Ar = Ph), was sometimes the major product when aqueous THF was used. **16** did not show any inhibition against PTP1B.

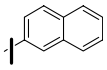
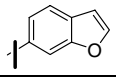
The X-ray co-crystal structure of **11** and PTP1B was successfully obtained (Fig. 4). It was found that the binding mode of **11** was similar to that of compound **2** (Fig. 3). The structural information has provided guidance for further optimization of this series of compounds. It was envisioned that substitution off the *para* position on the phenyl ring [7] would provide opportunities to capture interactions with Arg47 and Asp48 and thus enhance inhibition against PTP1B. In addition, the possibility of achieving modest selectivity against TCPTP by interacting with the residues in the YRD motif (residues 46–48) has been reported before.

Arg47 and Asp48 are close to the catalytic site, interaction of which has proven to be important in achieving inhibitor selectivity over most of the PTPs as reported by Zhang and co-workers.[8] The *para*-position on the C5-phenyl ring is 4.7 Å away from Asp48 (measured from the *para*-carbon of the phenyl to the acid moiety of Asp48) as revealed by X-ray co-crystal structure of **11** and PTP1B (Fig. 4). It was predicted by molecular modeling that a hydrogen bond would exist between the Asp48 and a donor at the *para*-position of the phenyl ring. In addition, it is also possible to introduce interactions with Arg47 that has been shown to significantly improve potency against PTP1B.[9]

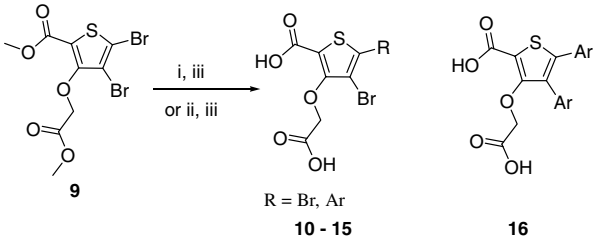
To confirm this hypothesis, compound **17** (Table 3) was synthesized according to Scheme 2. Indeed, a 10-fold increase in potency was quickly realized (Table 3). The existence of a hydrogen bond between the hydroxyl group of **17** and Asp48 was confirmed by X-ray crystallography (Fig. 5).

Table 2. The effect of C5-substitution on the inhibition against PTP1B



Compound	X	R	K_i[a] (μM)
10	Br	Br	18
11	Br		3.2
12	Br		1.4
13	Br		4.0
14	Br		3.3
15	Br		5.0

[a] See Table 1 for enzymatic assay conditions.



Scheme 2. Reagents and condition: (i) $Pd(PPh_3)_4$, $PhB(OH)_2$, KF, THF; (ii) $Pd(PPh_3)_4$, $PhB(OH)_2$, KF, THF/H_2O or dioxane/H_2O; (iii) LiOH, THF/H_2O, rt.

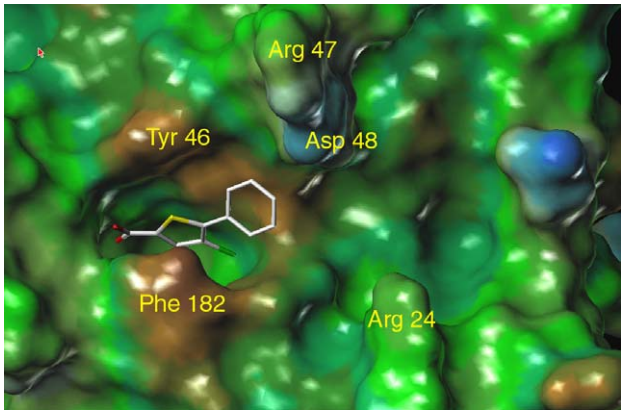
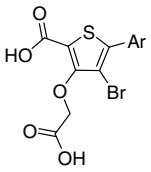
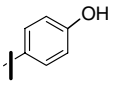
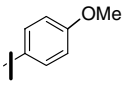
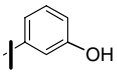
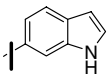


Figure 4. A molecular surface on X-ray co-crystal structure of compound **11** (PBD code: 2H4K). The surface is colored by lipophilicity with the more lipophilic regions shown in brown.

Table 3. The importance of a H-bond with Asp48



Compound	X	Ar	K_i[a] (μM)
17	Br		0.3
18	Br		3.0
19	Br		1.0
20	Br		0.57

[a] See Table 1 for enzymatic assay conditions.

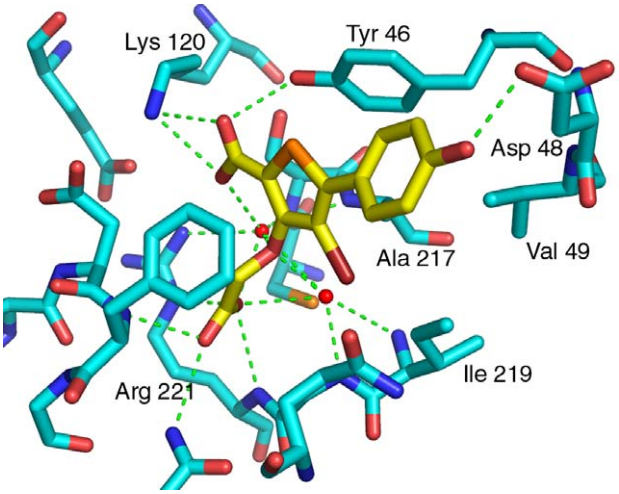
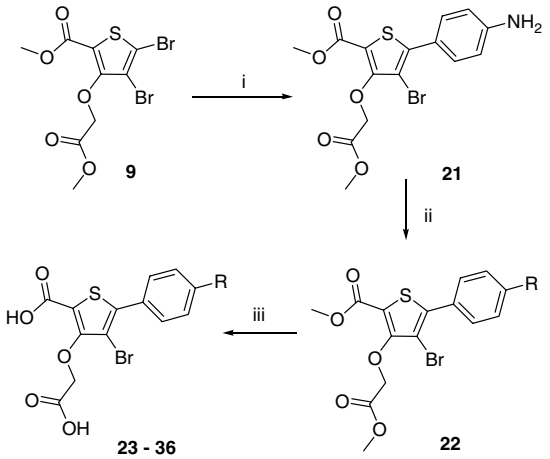
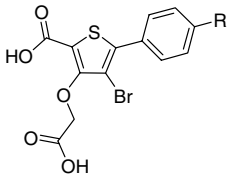


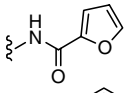
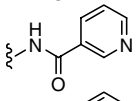
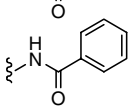
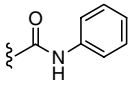
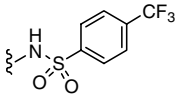
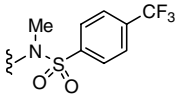
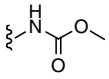
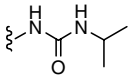
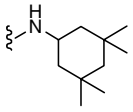
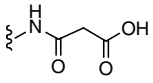
Figure 5. X-ray co-crystal structure of **17** with PTP1B (PBD code: 2H4G).



Scheme 3. Reagents: (i) $Pd(PPh_3)_4$, $ArB(OH)_2$, KF, THF/H_2O (70–78%); (ii) R′COCl, or R′SO_2Cl or R′–N=C=O or R′OCOCl, TEA, DCM (80–95%); (iii) LiOH, THF/H_2O (80–99%).

Table 4. The effect of the representative 5-*para*-substituted-phenyl thiophenes on the inhibition against PTP1B



Compound	R	K_i[a] (μM)
23	NH_2	1.6
24		9.0
25		0.50
26		0.62
27		0.82
28		0.60
29		2.5
30		0.74
31		0.20
32		1.2
33		1.0
34		0.36
35		0.25
36		0.14

[a] See Table 1 for enzymatic assay conditions.

Methylation of **17** in compound **18** reverted the potency back to that of **11** (Table 3), which reassured the importance of the hydrogen bond. Consistent with modeling prediction, a hydrogen-bond donor at the *meta*-position in compounds **19** and **20** was less effective likely due to less optimal directionality of hydrogen bond formation (Table 3).

Other H-bond donors have been subsequently adopted to further optimize the hydrogen bond interactions with Asp48 of PTP1B as well as balancing the overall physical–chemical properties. An array of amines, amides, sulfonamides, ureas, and carbamates were thus synthesized (Scheme 3 and Table 4). The importance of a hydrogen-bond interaction between the inhibitors and Asp48 was once again demonstrated. For instance, elimination of hydrogen-bond donor from **23** led to a more than 5-fold loss in potency as observed in compound **24**. Similarly, a 6-fold loss was observed in compound **32** compared to parent compound **31**. In contrast, increasing the acidity of the hydrogen-bond donor (NH group) strengthens its H-bond interaction with Asp48 and thus improves potency as in the case of amides (e.g., **25**–**27**), sulfonamides (e.g., **30**–**31**), carbamates (e.g., **33**), and ureas (e.g., **34**). Consistently, the reversed amide **29** is 4-fold less active than **28**, presumably due to the loss of the hydrogen bond between the amide NH and Asp48.

Incorporation of a tetramethylcyclohexyl group in **35** led to 6- to 7-fold increase in inhibition, likely resulting from additional van der Waals interactions with the relatively lipophilic region of the protein surface formed by the hydrocarbon part of the side chain of Asp48, Val 49, and Met258 as suggested by molecular modeling. A carboxylic group in **36** was designed to target electrostatic interaction with Arg47. Indeed, the presence of this carboxylic group resulted in significant improvement in inhibitory activity (compound **36**, K_i = 0.14 μM, Table 4).

These PTP1B inhibitors have shown good selectivity against other PTPases except for the highly homologous T-cell PTPase (TCPTP). Representative examples are shown in Table 5. In general, greater than 1000-fold of selectivity was observed against LAR (compounds **11**, **24**, **27**, and **36**, Table 5), and the selectivity against CD45 increases with the potency against PTP1B. For instance, more than 236-fold selectivity against CD45 was achieved with compound **36** ($K_{i(PTP1B)}$ = 0.14 μM, Table 5), while compound **24** ($K_{i(PTP1B)}$ = 0.3 μM, Table 5) only has a 40-fold selec-

Table 5. Inhibitory activity of select compounds against various protein tyrosine phosphatases*

Compound	PTP1b[a] K_i (μM)	TCPTP[b] K_i (μM)	CD45[c] K_i[d] (μM)	LAR[e] K_i (μM)
24	1.6	1.0	64 (40)	>1000
27	0.82	0.68	55 (67)	320
11	0.30	0.32	26 (87)	>1000
36	0.14	0.18	33 (236)	>1000

[a] PTP1B (residues 1–299) concentration: 20 nM.
[b] TCPTP (residues 1–299) concentration: 20 nM.
[c] CD45 (residues 484–1281, purchased from Biomol) concentration: 20 nM.
[d] Selectivity ratio is in parentheses.
[e] LAR (residues 1275–1613, purchased from Biomol) concentration: 20 nM.
* Enzymatic assay was carried out according to description on Table 1.

tivity against CD45. Though it has been known that interaction with the YRD motif could achieve modest selectivity against TCPTP,[8] there is no differentiation in inhibitory potency against PTP1B and TCPTP with this series of inhibitors.

In summary, a series of monocyclic thiophenes was discovered to be competitive and reversible PTP1B inhibitors with good potency and selectivity against other PTPases with the exception of TCPTP. A key hydrogen bond with Asp48 was proven to be pivotal in achieving better inhibition against PTP1B. This interaction was confirmed by X-ray co-crystal structures. Additional effort in further optimizing this monocyclic thiophene series will be reported in due course.

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