

Article

1*H*-2,3-Dihydroperimidine Derivatives: A New Class of Potent Protein Tyrosine Phosphatase 1B Inhibitors

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Abstract: A series of 1*H*-2,3-dihydroperimidine derivatives was designed, synthesized, and evaluated as a new class of inhibitors of protein tyrosine phosphatase 1B (PTP1B) with IC₅₀ values in the micromolar range. Compounds **46** and **49** showed submicromolar inhibitory activity against PTP1B, and good selectivity (3.48-fold and 2.10-fold respectively) over T-cell protein tyrosine phosphatases (TCPTP). These results have provided novel lead compounds for the design of inhibitors of PTP1B as well as other PTPs.

Keywords: 1*H*-2,3-dihydroperimidine derivatives; PTP1B; inhibitors; selectivity; structure-activity relationships (SAR)

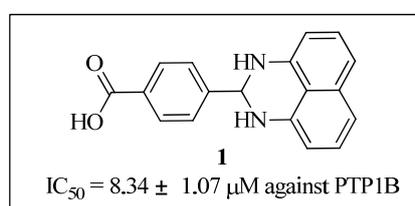
1. Introduction

Reversible protein tyrosine phosphorylation is a key regulatory mechanism in eukaryotic cell physiology [1]. Dysregulation of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) is linked to numerous human diseases, including cancer, diabetes, obesity, infection, autoimmune, and neuropsychiatric disorders [2,3]. Hence, PTKs and PTPs are emerging as high value targets for therapeutic intervention [3–7]. Consequently, many efforts have been made to target these enzymes with small molecules in order to develop new therapeutic agents. Notable success has been achieved in targeting signaling pathways regulated by protein tyrosine phosphorylation with more than a dozen of small molecule kinase inhibitors on the market [8]. However, the therapeutic benefits of modulating PTPs are still underexplored despite the fact that several PTPs have been identified as high value targets [9].

Protein tyrosine phosphatase 1B (PTP1B), an intracellular nonreceptor PTPase, has received much attention due to its pivotal role in type II diabetes and obesity as a negative regulator of the insulin signaling pathway by dephosphorylating the activated insulin receptor [10]. Studies from two different laboratories have shown PTP1B-knockout mice exhibit enhanced insulin sensitivity, improved glucose tolerance and resistance to diet-induced obesity [11,12]. In addition, several groups have demonstrated that overexpression of PTP1B is sufficient to drive tumorigenesis in mice, providing additional support for the use of PTP1B inhibitors for cancer therapy [13,14]. Therefore, PTP1B seems to be a potential target for the treatment of type 2 diabetes mellitus, obesity, and cancer. A variety of PTP1B inhibitors have been disclosed among academic and industrial laboratories [15–17]. Two compounds, ertiprotafib and trodusquemine, have progressed to clinical trials [18,19]. However, ertiprotafib was discontinued in phase II clinical trials due to a lack of efficacy and side effects [20]. There are two significant challenges to develop orally bioavailable, small molecular PTP1B inhibitors [15]: (1) it is difficult to design inhibitors that are specific for PTP1B due to the close homology with other PTPs, for example, T-cell protein tyrosine phosphatase (TCPTP) shares a structurally very similar active site with PTP1B and is about 80% homologous in the catalytic domain [21], and (2) many small molecules that bind with high affinity in active site are hydrophilic, and as a result they have poor cell permeability. Therefore, imminent development of potent and PTP1B specific inhibitor remains necessary.

In searching for novel PTP1B inhibitors, we identified 4-(2,3-dihydro-1*H*-perimidin-2-yl)benzoic acid (**1**) as a novel PTP1B inhibitor ($IC_{50} = 8.34 \pm 1.07 \mu\text{M}$) through high throughput screening of our compound collection (Figure 1). This result provided us a chance to explore novel small molecule inhibitors of PTP1B. Herein, we designed, synthesized a series of 1*H*-2,3-dihydroperimidine derivatives, evaluated their inhibitory activities toward PTP1B, and elucidated the SARs. Selected compounds were also subjected to selectivity analyses to determine whether their biological properties made them suitable for further development.

Figure 1. Chemical structure of hit compound **1**.



2. Results and Discussion

2.1. Chemistry

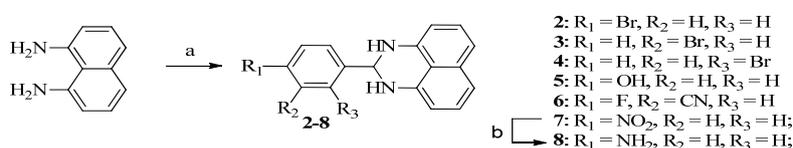
2.1.1. Design of 1*H*-2,3-Dihydroperimidine Derivatives

Based on the structure of compound **1**, **42** new 1*H*-2,3-dihydroperimidine derivatives (compounds **2–6**, **8–19**, **21–35**, and **40–49**, Table 1) were designed and synthesized. Replacing the carboxylic acid moiety in compound **1** with bromo-, hydroxyl-, fluoro-, cyano-, or amino groups, we obtained analogues **2–6** and **8**. By coupling the carboxylic acid on compound **1** with a series of amino acids esters, compounds **10–14** were obtained. After saponification, we got the corresponding acid compounds **15–19**. By replacing the amide bond in compounds **15–19** with an oxygen atom we got ether derivatives **20–25**. Using amine derivative **8** as starting material, amide compounds **26–35** were obtained. We also synthesized compounds **40–49** by replacement of phenyl ring on compound **1** with pyridinyl ring.

2.1.2. Synthesis of 1*H*-2,3-Dihydroperimidine Derivatives

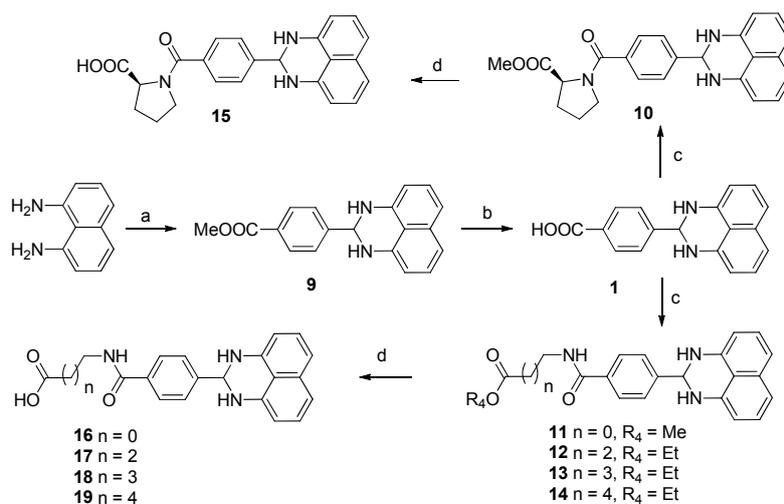
The coupling reactions of 1,8-diaminonaphthalene with various aldehydes were accomplished in the presence of a catalytic amount of Zn(OAc)₂ to yield compounds **2–7** (Scheme 1) and **9** (Scheme 2) in 31%–55% yields [22]. After reduction of nitro compound **7**, amine **8** was obtained in 89% yield. Compound **1** was obtained from compound **9** in 53% by saponification with lithium hydroxide in aqueous THF, followed by coupling with appropriate amino acid esters to yield compounds **10–14** in 35%–52% yield. After saponification with lithium hydroxide in aqueous THF, compounds **15–19** were obtained in 42%–55% yield (Scheme 2). Compounds **20–22** were prepared by alkylation of the phenol **5** with a series of bromo ethyl esters, followed by saponification with lithium hydroxide in aqueous THF to yield **23–25** in 35%–54% yield (Scheme 3). Amide compounds **26–29** were obtained in 35%–51% yield by acylation with ethyl chlorooxoacetate or various ethyl ester acids, followed by saponification with lithium hydroxide in aqueous THF to yield **30–33** in 35%–56% yield (Scheme 4). Compound **34** was prepared by coupling amine **8** with 3-((*tert*-butoxycarbonyl)amino)propanoic acid in the presence of EDCI and DMAP, followed by deprotection of the Boc group using TFA in CH₂Cl₂ to yield the amino compound **35** (Scheme 5). Scheme 6 describes the straightforward synthesis of the derivatives **40–49**. Obtained from compound **36** in the presence of SOCl₂ using MeOH as solvent, diester **37** was selectively reduced to compound **38** in the presence of NaBH₄/CaCl₂ [23]. Compound **38** was transformed into aldehyde **39** using Dess-Martin periodinane in the presence of CH₂Cl₂, followed by coupling with naphthalene-1,8-diamine to yield compound **40**, using similar conditions to those in Scheme 1. Then compounds **41–49** were obtained using similar conditions to those shown in Scheme 2.

Scheme 1. Synthesis of Compounds **2–8**.



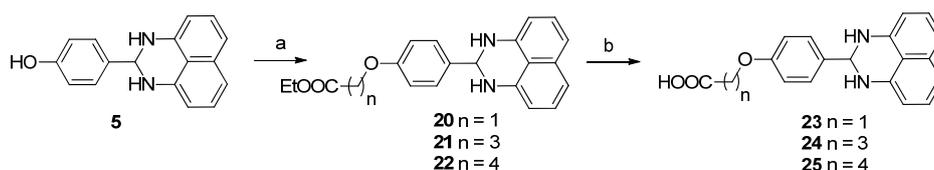
Reagents and conditions: a—MeOH, Zn(OAc)₂, aldehyde, overnight, 45%–55%; b—Fe, NH₄Cl, EtOH/H₂O, 89%.

Scheme 2. Synthesis of Compounds 10–19.



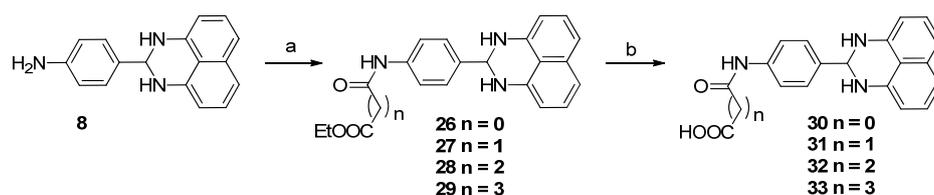
Reagents and conditions: a—MeOH, $\text{Zn}(\text{OAc})_2$, 4-formylbenzoic acid methyl ester, overnight, 31%; b—LiOH, THF/ H_2O , 3 h, 53%; c—amino acid esters, EDCI, DMAP, 40 °C, overnight, 35%–52%; d—LiOH, THF/ H_2O , 42%–55%.

Scheme 3. Synthesis of Compounds 20–25.



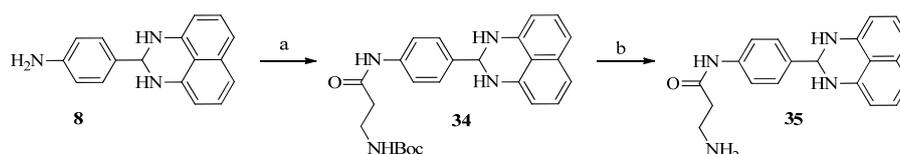
Reagents and Conditions: a— $\text{Br}(\text{CH}_2)_n\text{COOEt}$, Cs_2CO_3 , 40 °C overnight, 40%–65%; b—LiOH, THF/ H_2O , 35%–54%.

Scheme 4. Synthesis of Compounds 26–33.



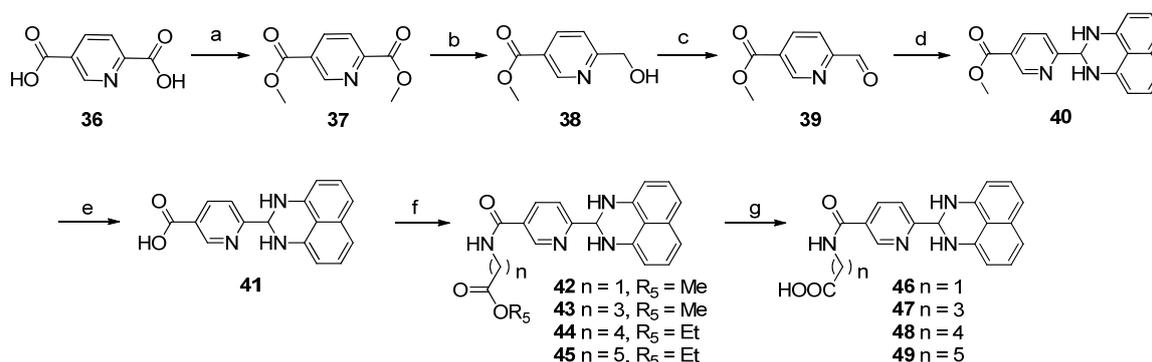
Reagents and Conditions: a— ClCOCOOEt , Et_3N , DCM, 41% or $\text{EtOCO}(\text{CH}_2)_n\text{COOH}$, EDC, DMAP, DMF, 35%–51%; b—LiOH, THF/ H_2O , 35%–56%.

Scheme 5. Synthesis of Compounds 34–35.



Reagents and Conditions: a— $\text{BocNH}(\text{CH}_2)_2\text{COOH}$, EDCI, DMAP, DMF, 43%; b—TFA, DCM, 67%.

Scheme 6. Synthesis of Compounds 40–49.



Reagents and Conditions: a—MeOH, SOCl₂, 70 °C, 4 h, 98%; b—CaCl₂, NaBH₄, THF/EtOH, 0 °C, 58%;
 c—Dess-Martin periodinane, DCM, overnight, 86%; d—naphthalene-1,8-diamine, MeOH, Zn(OAc)₂,
 overnight, 27%; e—LiOH, THF/H₂O, 43%; f—NH₂(CH₂)_nCOOR₅, EDCI, DMAP, DMF, 40%–60%;
 g—LiOH, THF/H₂O, 42%–50%.

2.2. Biological Activities

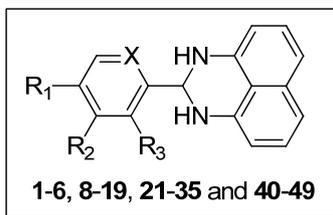
2.2.1. PTP1B Inhibitory Activities and Structure-Activity Relationships

The inhibitory activities of all the synthesized compounds against PTP1B were measured using *p*-nitrophenyl phosphate (pNPP) as substrate [24,25], and the results are detailed in Table 1. We initially prepared 2–6 and 8 by the route outlined in Scheme 1, in which we substituted the carboxylic acid group on the phenyl ring with bromo-, fluoro-, hydroxyl-, amino-, or cyano groups. We noticed that none of them showed more than 50% of enzyme inhibition against PTP1B at the concentration of 20 µg/mL. This result indicated the importance of the carboxylic acid for activity. As for compounds 9–14, ester compounds showed poor enzyme inhibitory activity at the concentration of 20 µg/mL. Saponification of ester compounds 9–14 to the corresponding acid compounds 1 and 15–19 dramatically improved PTP1B inhibitory activity. These results further confirmed that the acid group was important for PTP1B inhibitory activity. Among compound 1 and 16–19, compound 16 with an acetic acid moiety and compound 17 with a butanoic acid moiety showed three times less potency than compound 1. Compound 18 with a pentanoic acid moiety and compound 19 with a hexanoic acid moiety showed similar activity to compound 1. Compound 15 with a proline moiety exhibited similar activity to compound 16. The results indicated that the distance between phenyl ring and carboxylic acid had some impact on the inhibitory activity. As for other compounds 21–25, ester compounds 21 and 22 did not show inhibitory activity.

Compound 23 with an acetic acid moiety exhibited slightly better inhibitory activity than compound 1. However, compound 24 with butanoic acid moiety and compound 25 with pentanoic acid moiety showed no inhibitory activity. These results indicated that using O atom as linker between phenyl ring and carboxylic acid generally decreased the enzyme inhibitory activity and that the distance between carboxylic acid and phenyl ring impacted the enzyme inhibitory activity. As for amide compounds 26–33, ester compounds 27–29 did not show activity, however it was interesting that compound 26 with an ethyl 2-oxoacetate moiety showed six times more potent enzyme inhibitory activity than compound 1. Among acid compounds 30–33, compound 30 with a 2-oxoacetic acid moiety and compound 32 with a

4-oxobutanoic acid had no activity against PTP1B. Compound **31** with a 3-oxopropanoic acid and compound **33** with a 5-oxopentanoic acid showed similar activity to compound **1**. Compound **34** and **35** showed no inhibitory activity against PTP1B.

Table 1. PTP1B inhibitory activities of compounds **1–6**, **8–19**, **21–35** and **40–49**.



Comp	R ₁	R ₂	R ₃	X	Inhibition(%) at 20 µg/mL	IC ₅₀ (µM) ^a
1	COOH	H	H	CH	98.63%	8.34 ± 1.07
2	Br	H	H	CH	37.00%	NT ^b
3	H	Br	H	CH	42.51%	NT
4	H	H	Br	CH	17.52%	NT
5	OH	H	H	CH	7.18%	NT
6	F	CN	H	CH	31.58%	NT
8	NH ₂	H	H	CH	23.76%	NT
9	COOMe	H	H	CH	15.27%	NT
10		H	H	CH	26.25%	NT
11		H	H	CH	4.81%	NT
12		H	H	CH	6.83%	NT
13		H	H	CH	28.48%	NT
14		H	H	CH	35.57%	NT
15		H	H	CH	84.21%	20.23 ± 1.94
16		H	H	CH	89.99%	27.75 ± 5.45
17		H	H	CH	73.13%	22.21 ± 1.60
18		H	H	CH	94.84%	5.53 ± 0.54
19		H	H	CH	95.35%	7.82 ± 0.27
21		H	H	CH	7.45%	NT
22		H	H	CH	2.04%	NT
23		H	H	CH	98.39%	5.88 ± 0.25
24		H	H	CH	10.48%	NT

Table 1. Cont.

Comp	R ₁	R ₂	R ₃	X	Inhibition(%) at 20 µg/mL	IC ₅₀ (µM) ^a
25		H	H	CH	32.13%	NT
26		H	H	CH	91.76%	1.27 ± 0.06
27		H	H	CH	1.68%	NT
28		H	H	CH	29.69%	NT
29		H	H	CH	2.92%	NT
30		H	H	CH	0.51%	NT
31		H	H	CH	96.83%	6.45 ± 0.42
32		H	H	CH	29.95%	NT
33		H	H	CH	95.07%	6.91 ± 1.17
34		H	H	CH	12.61%	NT
35		H	H	CH	0.29%	NT
40	COOMe	H	H	N	6.40%	NT
41	COOH	H	H	N	96.62%	10.82 ± 0.69
42		H	H	N	42.12%	NT
43		H	H	N	4.67%	NT
44		H	H	N	4.74%	NT
45		H	H	N	10.75%	NT
46		H	H	N	99.47%	0.66 ± 0.03
47		H	H	N	98.47%	15.24 ± 1.41
48		H	H	N	91.47%	3.56 ± 0.13
49		H	H	N	99.40%	0.59 ± 0.05
OA ^c	-	-	-	-	-	2.41 ± 0.35

^a The pNPP assay. IC₅₀ values were determined by regression analyses and expressed as means ± SD of three replications; ^b NT means not tested; ^c OA means oleanolic acid as positive control.

By replacing phenyl ring on compound **1** with a pyridinyl ring, ester compounds **40** and **42–45** did not show inhibitory activity. As for acid compounds, compound **41** showed similar activity to hit compound **1**. Compound **46** with acetic acid moiety and compound **49** with a hexanoic acid moiety showed submicromolar inhibitory activity, about fourteen times more potent than compound **1**. Compound **48** with a pentanoic acid moiety had inhibitory activity with IC_{50} of $3.56 \pm 0.13 \mu\text{M}$. Compound **47** with a butanoic acid moiety showed poorest inhibitory activity with IC_{50} of $15.24 \pm 1.41 \mu\text{M}$, about twenty-five times less potent than compounds **46** and **49**. The results indicated that the distance between pyridinyl ring and carboxylic acid was important for enzyme inhibitory activity, and that the replacement of phenyl ring with a pyridinyl ring obviously impacted on the enzyme inhibition.

2.2.2. Selectivity against Other PTPs

In addition to potency improvements, we investigated the selectivity of three representative compounds, namely, **1**, **46** and **49** against other PTPs (TCPTP, SHP-1, SHP-2, LAR). As shown in Table 2, homogeneous T-cell protein tyrosine phosphatase (TCPTP) inhibitory activities were investigated simultaneously by the same method [24,25]. Compounds **46** and **49** showed 3.48-fold and 2.10-fold greater selectivity for PTP1B than for TCPTP respectively, while hit compound **1** exhibited poor selectivity with 0.58-fold for PTP1B than for TCPTP. Besides TCPTP, we tested the inhibitory activity of these compounds on other three homogenous enzymes SHP-1, SHP-2, and LAR [25]. As shown in Table 2, we concluded that compounds **46** and **49** had no visible activities against LAR, and compound **46** possessed about 9-fold selectivity for PTP1B over SHP-1 and SHP-2, while **49** showed about 4-fold selectivity for PTP1B over SHP-1 and SHP-2.

Table 2. The IC_{50} values of compounds **1**, **46** and **49** against PTPs.

Comp	$IC_{50}(\mu\text{M})^a$		TCPTP/PTP1B	$IC_{50}(\mu\text{M})^a$		
	PTP1B	TCPTP		SHP-1	SHP-2	LAR
1	8.34 ± 1.07	4.83 ± 0.90	0.58	23.31 ± 2.03	31.21 ± 7.72	NA ^b
46	0.66 ± 0.03	2.30 ± 0.17	3.48	6.01 ± 0.20	5.95 ± 0.29	NA
49	0.59 ± 0.05	1.24 ± 0.12	2.10	2.72 ± 0.30	2.50 ± 0.22	NA
PC ^c	2.41 ± 0.35	5.14 ± 0.77		58.34 ± 1.96	36.65 ± 4.46	58.34 ± 1.96

SHP-1, SH2-Containing Protein Tyrosine Phosphatase-1; SHP-2, SH2-Containing Protein Tyrosine Phosphatase-2; LAR, leukocyte antigen-related tyrosine phosphatase; ^a The *p*NPP substrate and 3-o-methylfluorescein phosphate (OMFP) substrate were utilized in PTP1B/TCPTP assay, and SHP-1/SHP-2/LAR assay, respectively; IC_{50} values were determined by regression analyses and expressed as means \pm SD of three replications; ^b NA: No activity (compound inhibitory ratio lower than 50% at the dose of 20 $\mu\text{g}/\text{mL}$); ^c PC: positive control; Oleonic acid was for PTP1B and TCPTP, Na_3VO_4 was for SHP-1, SHP-2 and LAR.

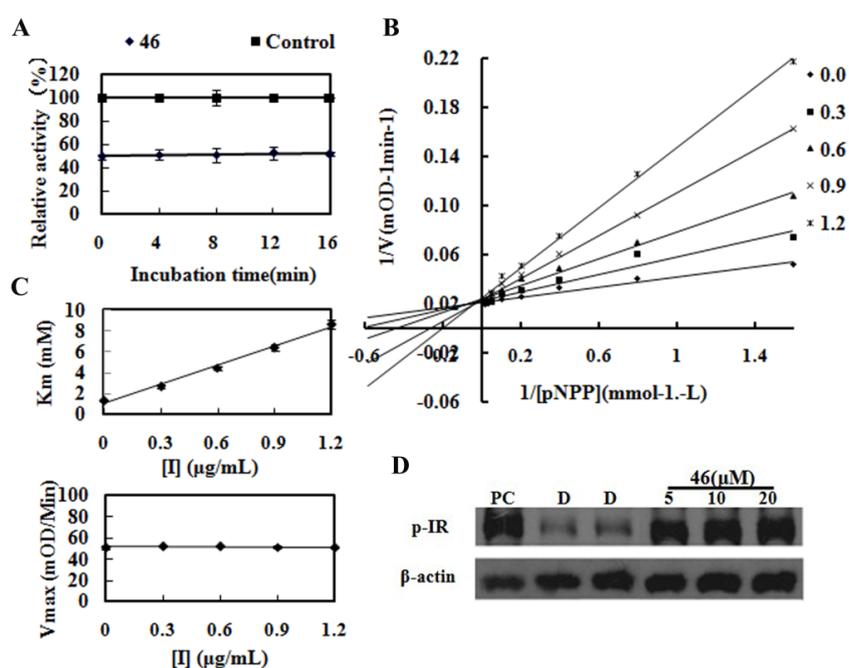
2.2.3. Characterization of the Inhibitor on Enzyme Kinetics and Cellular Activity

A kinetic study was performed in order to identify the inhibitory mechanism of compound **46** (Figure 2), using the reported enzyme kinetics assays [25].

As shown in Figure 2A, **46** demonstrated a fast-binding inhibition of PTP1B. The fast-binding inhibition of **46** toward PTP1B may also exclude that **46** is a nonspecific inhibitor, because nonspecific inhibitors always show time-dependent behavior and steep inhibition curve [26]. We further

determined the inhibition modality of **46** which inhibited PTP1B with the characteristics typical of a competitive inhibitor, as indicated by increased k_m values and unchanged V_{max} values when the inhibitor concentration was increased (Figure 2C). Meanwhile, the result of the Lineweaver-Burk plot confirmed **46** as a competitive inhibitor of PTP1B for intersecting at y-axis of a nest of lines with increased inhibitor concentration (Figure 2B). The results indicated that **46** binds the catalytic pocket of PTP1B and behaves as a competitor to the substrate.

Figure 2. Characterization of **46** to PTP1B. (A) Fast-binding inhibition of PTP1B by **46**; (B) Typical competitive inhibition of **46** shown by Lineweaver-Burk plot; (C) The initial velocity determined with various concentrations of pNPP at various fixed concentrations of **46**; and (D) Effect of **46** on tyrosine phosphorylation of insulin receptor β in CHO/*hIR* cells.



To explore the effect of PTP1B inhibitor *in vitro* on insulin signaling, CHO cells overexpressing human insulin receptor (CHO/*hIR*) were treated in presence or absence of compound, then tyrosine phosphorylation level of insulin receptor (p-IR) was detected after stimulated by insulin. Compared with negative control of DMSO, compound **46** ranging from 5 μ M to 20 μ M greatly improved p-IR level. This result indicates that compound **46** shows well membrane permeability and could protect insulin pathway signaling on the cellular level (Figure 2D).

3. Experimental

3.1. Chemistry

All chemicals were reagent grade and used as purchased. All reactions were performed under an inert atmosphere of dry argon or nitrogen using distilled dry solvent. ^1H (400 MHz) NMR spectra were recorded on a Bruker AVIII 400 MHz spectrometer. The chemical shifts were reported in (ppm) using the 7.26 signal of CDCl_3 (^1H -NMR) and the 2.50 signal of $\text{DMSO-}d_6$ (^1H -NMR) as internal standards and the 39.50 signal of $\text{DMSO-}d_6$ (^{13}C -NMR) as internal standards. ESI Mass spectra (MS) were

obtained on a Waters Micromass Platform LCZ Mass Spectrometer. Melting points were recorded on YRT-3 melting point apparatus (Tianjin Reliant Instrument Co., Ltd., Tianjin, China) and were reported without correction.

3.1.1. Procedure for the Preparation of Compounds 2–7

To a stirred solution of 4-bromobenzaldehyde (421.8 mg, 2.28 mmol) in methanol (5 mL) was added a solution of naphthalene-1,8-diamine (300 mg, 1.90 mmol) in methanol (5 mL), followed by Zn(OAc)₂·H₂O (3.5 mg, 0.016 mmol). Then the mixture was stirred at room temperature for 16 h. The reaction mixture was filtered. The filter cake was washed with methanol, dried to get compound **2** (277 mg, 45%) as a brown solid, mp 138.9–142.3 °C. ¹H-NMR (CDCl₃) δ: 4.68 (brs, 2H), 5.45 (s, 1H), 6.54 (dd, *J* = 1.6 Hz, 6.4 Hz, 2H), 7.25(m, 4H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 2H); MS (ESI): *m/z* calcd. for C₁₇H₁₄BrN₂[M+H]⁺ 325.0/327.0, found: 325.2/327.5.

2-(3-Bromophenyl)-2,3-dihydro-1H-perimidine (3). Yield = 48%, mp 161.7–163.3 °C; ¹H-NMR (CDCl₃) δ: 4.51 (brs, 2H), 5.44 (s, 1H), 6.54 (dd, *J* = 1.6 Hz, 6.8 Hz, 2H), 7.23–7.34 (m, 5H), 7.55–7.59 (m, 2H), 7.83 (s, 1H); MS (ESI): *m/z* calcd. for C₁₇H₁₄BrN₂ [M+H]⁺ 325.0/327.0, found: 325.5/327.5.

2-(2-Bromophenyl)-2,3-dihydro-1H-perimidine (4). Yield = 46%, mp 130.4–131.0 °C; ¹H-NMR (CDCl₃) δ: 4.62 (s, 2H), 5.94 (s, 1H), 6.58 (d, *J* = 7.2 Hz, 2H), 7.23–7.30 (m, 5H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.84 (dd, *J* = 1.2 Hz, 7.6 Hz, 1H); MS (ESI): *m/z* calcd. for C₁₇H₁₄BrN₂ [M+H]⁺ 325.0/327.0, found: 325.4/327.7.

4-(2,3-Dihydro-1H-perimidin-2-yl)phenol (5). Yield = 52%, mp 148.1–152.2 °C; ¹H-NMR (DMSO-*d*₆) δ: 5.25 (s, 1H), 6.48 (d, *J* = 7.2 Hz, 2H), 6.62 (s, 2H), 6.82 (d, *J* = 8.0 Hz, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 7.14 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.41(d, *J* = 8.4 Hz, 2H), 9.54 (s, 1H); MS (ESI): *m/z* calcd. for C₁₇H₁₅N₂O[M+H]⁺ 263.1, found: 263.2.

5-(2,3-Dihydro-1H-perimidin-2-yl)-2-fluorobenzonitrile (6). Yield = 55%, mp 204.4–208.4 °C; ¹H-NMR (DMSO-*d*₆) δ: 5.44 (s, 1H), 6.50 (d, *J* = 7.6 Hz, 2H), 6.90 (s, 2H), 7.01(d, *J* = 7.6 Hz, 2H), 7.17 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.60 (t, *J* = 9.2 Hz, 1H), 7.99 (dt, *J* = 2.4 Hz, 6.8 Hz, 1H), 8.14 (dd, *J* = 2.4 Hz, 6.4 Hz, 1H); MS (ESI): *m/z* calcd. for C₁₈H₁₃FN₃[M+H]⁺ 290.1, found: 290.3.

2-(4-Nitrophenyl)-2,3-dihydro-1H-perimidine (7). Yield = 54%; ¹H-NMR (DMSO-*d*₆) δ: 5.50 (s, 1H), 6.52 (d, *J* = 7.2 Hz, 2H), 6.99 (s, 2H), 7.01 (d, *J* = 8.4 Hz, 2H), 7.18 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.85 (d, *J* = 8.8 Hz, 2H), 8.28 (d, *J* = 8.8 Hz, 2H); MS (ESI): *m/z* calcd. for C₁₇H₁₄N₃O₂ [M+H]⁺ 292.1, found: 292.5.

3.1.2. Procedure for the Preparation of Compound 8

The mixture of compound **7** (100 mg, 0.34 mmol), iron (38.5 mg, 0.69 mmol) and NH₄Cl (55.2 mg, 1.03 mmol) in the solution of ethanol (2 mL) and water (1 mL) was heated at 90 °C for 3 h. After filtration, the filter cake was washed with EtOAc, concentrated the filtrate, and dried to afford compound **8** (80 mg, 89%), mp 166.4–171.9 °C. ¹H-NMR (DMSO-*d*₆) δ: 5.17 (s, 1H), 6.47 (d, *J* = 8.0 Hz,

2H), 6.53 (s, 2H), 6.62 (d, $J = 8.0$ Hz, 2H), 6.96 (d, $J = 8.0$ Hz, 2H), 7.13 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.26 (d, $J = 8.0$ Hz, 2H); MS (ESI): m/z calcd. for $C_{17}H_{16}N_3[M+H]^+$ 262.1, found: 262.1.

3.1.3. General Procedure for the Preparation of Derivatives 9–19

To a stirred solution of naphthalene-1,8-diamine (500 mg, 3.16 mmol) in methanol (10 mL) was added a solution of 4-formylbenzoic acid methyl ester (621.6 mg, 3.79 mmol) in methanol (5 mL), followed by $Zn(OAc)_2$ (58.2 mg, 0.26 mmol). The mixture was stirred at room temperature for 16 h. After filtration, the filter cake was washed with methanol, dried to give compound **9** (300 mg, 31%), mp 165.0–168.2 °C. 1H -NMR ($CDCl_3$) δ : 3.95 (s, 3H), 4.52 (s, 2H), 5.54 (s, 1H), 6.56 (dd, $J = 1.6$ Hz, 6.8 Hz, 2H), 7.24–7.30 (m, 4H), 7.72 (d, $J = 8.0$ Hz, 2H), 8.11 (d, $J = 8.0$ Hz, 2H); MS (ESI): m/z calcd. for $C_{19}H_{17}N_2O_2[M+H]^+$ 305.1, found: 305.2.

$LiOH \cdot H_2O$ (43.5 mg, 0.99 mmol) was added to a solution of compound **9** (100 mg, 0.33 mmol) in THF (1 mL)/ H_2O (1 mL). The mixture was stirred at room temperature for 3 h. After removal of THF, the water layer was washed with EtOAc, and acidified with HCl (1 M) to pH = 2, filtered and dried to get compound **1** (50 mg, 53%), mp > 265 °C; 1H -NMR ($DMSO-d_6$) δ : 5.45 (s, 1H), 6.51 (d, $J = 7.2$ Hz, 2H), 6.87 (s, 2H), 7.00 (d, $J = 8.0$ Hz, 2H), 7.17 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.72 (d, $J = 8.4$ Hz, 2H), 7.99 (d, $J = 8.0$ Hz, 2H), 12.93 (brs, 1 H); MS (ESI): m/z calcd. for $C_{18}H_{15}N_2O_2[M+H]^+$ 291.1, found: 291.0.

To a stirred solution of compound **1** (1.0 g, 3.4 mmol) in DMF (10 mL) was added methyl glycinate (0.3 g, 3.4 mmol), followed by EDCI (1.0 g, 5.2 mmol) and DMAP (0.04 g, 0.34 mmol). The mixture was stirred at 40 °C overnight. The reaction was diluted with EtOAc (100 mL), washed with water (200 mL \times 3). The combined organic phases were then processed in the usual way and chromatographed (2:1 petroleum ether/EtOAc) to yield compound **11** (0.5 g, 40%), mp 193.3–198.7 °C; 1H -NMR ($DMSO-d_6$) δ : 3.68 (s, 3H), 4.04 (d, $J = 6.0$ Hz, 2H), 5.44 (s, 1H), 6.51 (d, $J = 7.6$ Hz, 2H), 6.85 (s, 2H), 7.00 (d, $J = 8.0$ Hz, 2H), 7.17 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.70 (d, $J = 8.0$ Hz, 2H), 7.92 (d, $J = 8.4$ Hz, 2H), 8.99 (t, $J = 6.4$ Hz, 1H); MS (ESI): m/z calcd. for $C_{21}H_{20}N_3O_3[M+H]^+$ 362.2, found: 362.1.

$LiOH \cdot H_2O$ (69.8 mg, 1.66 mmol) was added to a solution of compound **11** (200 mg, 0.55 mmol) in THF (2 mL)/ H_2O (2 mL). The reaction was stirred at room temperature for 3 h. After removal of THF, the water layer was washed with EtOAc, acidified with HCl (1 M) to pH = 2, filtered and dried to afford compound **16** (80 mg, 42%), mp 85.4–90.3 °C; 1H -NMR ($DMSO-d_6$) δ : 3.93 (d, $J = 6.0$ Hz, 2H), 5.42 (s, 1H), 6.50 (d, $J = 7.2$ Hz, 2H), 6.83 (s, 2H), 6.98 (d, $J = 8.0$ Hz, 2H), 7.17 (t, $J = 7.6$ Hz, 2H), 7.68 (d, $J = 8.4$ Hz, 2H), 7.90 (d, $J = 8.4$ Hz, 2H), 8.86 (t, $J = 6.0$ Hz, 1H), 12.60 (brs, 1H); ^{13}C -NMR ($DMSO-d_6$) δ : 41.2, 65.7, 104.4, 112.0, 115.3, 126.8, 127.2, 127.7, 133.9, 134.3, 142.7, 145.1, 166.7, 171.2; MS (ESI): m/z calcd. for $C_{20}H_{18}N_3O_3[M+H]^+$ 348.1, found: 348.5.

Methyl 1-(4-(2,3-dihydro-1H-perimidin-2-yl)benzoyl)pyrrolidine-2-carboxylate (10). Yield = 41%, mp 171.3–176.0 °C; 1H -NMR ($CDCl_3$) δ : 1.93 (m, 1H), 2.04 (m, 2H), 2.34 (m, 1H), 3.55 (m, 1H), 3.64 (m, 1H), 3.80 (s, 3H), 4.53 (s, 2H), 4.69 (dd, $J = 4.8$ Hz, 8.4 Hz, 1H), 5.51 (s, 1H), 6.55 (d, $J = 6.8$ Hz, 2H), 7.27 (m, 4H), 7.65 (d, $J = 8.0$ Hz, 2H), 7.69 (d, $J = 8.0$ Hz, 2H); MS (ESI): m/z calcd. for $C_{24}H_{24}N_3O_3[M+H]^+$ 402.2, found: 401.7.

Ethyl 4-(4-(2,3-dihydro-1H-perimidin-2-yl)benzamido)butanoate (12). Yield = 35%, mp 128.6–132.5 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.16 (t, *J* = 7.2 Hz, 3H), 1.78 (m, 2H), 2.35 (t, *J* = 7.2 Hz, 2H), 3.27 (m, 2H), 4.04 (q, *J* = 7.2 Hz, 2H), 5.42 (s, 1H), 6.49 (d, *J* = 8.0 Hz, 2H), 6.83 (s, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 7.15 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 8.0 Hz, 2H), 8.50 (t, *J* = 5.2 Hz, 1H); MS (ESI): *m/z* calcd. for C₂₄H₂₆N₃O₃ [M+H]⁺ 404.2, found: 404.0.

Ethyl 5-(4-(2,3-dihydro-1H-perimidin-2-yl)benzamido)pentanoate (13). Yield = 52%, mp 141.8–143.1 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.17 (t, *J* = 7.2 Hz, 3H), 1.54 (m, 4H), 2.32 (t, *J* = 6.8 Hz, 2H), 3.26 (m, 2H), 4.04 (q, *J* = 7.2 Hz, 2H), 5.41 (s, 1H), 6.49 (d, *J* = 7.6 Hz, 2H), 6.83 (s, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 7.15 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.86 (d, *J* = 8.0 Hz, 2H), 8.48 (t, *J* = 5.2 Hz, 1H); MS (ESI): *m/z* calcd. for C₂₅H₂₈N₃O₃ [M+H]⁺ 418.2, found: 418.1.

Ethyl 6-(4-(2,3-dihydro-1H-perimidin-2-yl)benzamido)hexanoate (14), Yield = 48%, mp 145.8–147.4 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.16 (t, *J* = 7.2 Hz, 3H), 1.23–1.34 (m, 2H), 1.48–1.57 (m, 4H), 2.28 (t, *J* = 7.2 Hz, 2H), 3.23 (m, 2H), 4.04 (q, *J* = 7.2 Hz, 2H), 5.42 (s, 1H), 6.49 (d, *J* = 7.6 Hz, 2H), 6.83 (s, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 7.15 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.86 (d, *J* = 8.0 Hz, 2H), 8.46 (t, *J* = 5.2 Hz, 1H); MS (ESI): *m/z* calcd. for C₂₆H₃₀N₃O₃ [M+H]⁺ 432.2, found: 432.2.

1-(4-(2,3-Dihydro-1H-perimidin-2-yl)benzoyl)pyrrolidine-2-carboxylic acid (15). Yield = 42%, mp 233.1–234.3 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.91 (m, 4H), 2.29 (m, 2H), 4.42 (m, 1H), 5.42 (s, 1H), 6.51 (d, *J* = 8.0 Hz, 2H), 6.84 (s, 2H), 7.00 (d, *J* = 8.0 Hz, 2H), 7.16 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.67 (d, *J* = 8.0 Hz, 2H); MS (ESI): *m/z* calcd. for C₂₃H₂₂N₃O₃ [M+H]⁺ 388.2, found: 388.2.

4-(4-(2,3-Dihydro-1H-perimidin-2-yl)benzamido)butanoic acid (17). Yield = 55%, mp 99.7–103.3 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.75 (m, 2H), 2.27 (m, 2H), 3.27 (m, 2H), 5.42 (s, 1H), 6.49 (d, *J* = 7.2 Hz, 2H), 6.83 (s, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 7.15 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.87 (d, *J* = 8.0 Hz, 2H), 8.51 (t, *J* = 5.2 Hz, 1H); ¹³C-NMR (DMSO-*d*₆) δ: 24.5, 31.2, 38.7, 65.7, 104.4, 112.4, 115.3, 126.8, 127.1, 127.6, 134.3, 134.6, 142.7, 144.8, 165.6, 174.2; MS (ESI): *m/z* calcd. for C₂₂H₂₂N₃O₃ [M+H]⁺ 376.2, found: 376.1.

5-(4-(2,3-Dihydro-1H-perimidin-2-yl)benzamido)pentanoic acid (18). Yield = 47%, mp 102.8–107.4 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.55 (m, 4H), 2.22–2.28 (m, 2H), 3.25 (m, 2H), 5.42 (s, 1H), 6.50 (d, *J* = 7.2 Hz, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 7.15 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 8.0 Hz, 2H), 8.49 (t, *J* = 5.2 Hz, 1H); ¹³C-NMR (DMSO-*d*₆) δ: 22.0, 28.6, 33.3, 38.8, 65.8, 104.7, 112.6, 115.6, 126.8, 127.0, 127.7, 134.3, 134.7, 142.4, 144.4, 165.7, 174.4; MS (ESI): *m/z* calcd. for C₂₃H₂₄N₃O₃ [M+H]⁺ 390.2, found: 390.1.

6-(4-(2,3-Dihydro-1H-perimidin-2-yl)benzamido)hexanoic acid (19). Yield = 50%, mp 90.8–91.9 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.30 (m, 2H), 1.48–1.56 (m, 4H), 2.21 (t, *J* = 7.2 Hz, 2H), 3.24 (m, 2H), 5.41 (s, 1H), 6.49 (d, *J* = 8.0 Hz, 2H), 6.83 (s, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 7.15 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.86 (d, *J* = 8.4 Hz, 2H), 8.46 (t, *J* = 5.2 Hz, 1H); ¹³C-NMR (DMSO-*d*₆) δ: 24.2, 26.0, 28.8, 33.6, 39.0, 65.7, 104.4, 112.5, 115.3, 126.8, 127.0, 127.6, 134.3, 134.7, 142.7, 144.7, 165.7, 174.4; MS (ESI): *m/z* calcd. for C₂₄H₂₆N₃O₃ [M+H]⁺ 404.2, found: 404.5.

3.1.4. General Procedure for the Preparation of Derivatives 21–25

To a stirred solution of compound **5** (50 mg, 0.19 mmol) in DMF (2 mL) was added ethyl 4-bromobutyrate (44.6 mg, 0.23 mmol) and cesium carbonate (74.9 mg, 0.23 mmol). Then the resulting mixture was stirred at 40 °C overnight. The reaction was diluted with EtOAc (100 mL), washed with water (100 mL × 3). The organic phase was processed in the usual way and chromatographed (1:1 petroleum ether/EtOAc) to yield compound **21** (41 mg, 65%), mp 76.2–78.6 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.18 (t, *J* = 7.2 Hz, 3H), 1.96 (t, *J* = 6.4 Hz, 2H), 2.45 (m, 2H), 4.00 (t, *J* = 6.4 Hz, 2H), 4.07 (q, *J* = 7.2 Hz, 2H), 5.29 (s, 1H), 6.47 (d, *J* = 7.2 Hz, 2H), 6.66 (s, 2H), 6.96 (d, *J* = 8.0 Hz, 4H), 7.13 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H); MS (ESI): *m/z* calcd. for C₂₃H₂₅N₂O₃ [M+H]⁺ 377.2, found: 377.6.

The mixture of LiOH·H₂O (67.0 mg, 1.60 mmol) and compound **21** (200 mg, 0.53 mmol) in THF (2 mL) and H₂O (2 mL) was stirred at room temperature for 3 h. After removal of THF, the water layer was washed with EtOAc, acidified with HCl (1 M) to pH 2, filtered and dried to yield compound **24** (100 mg, 54%), mp 126.3–130.9 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.94 (m, 2H), 2.39 (t, *J* = 7.2 Hz, 2H), 4.02 (t, *J* = 7.2 Hz, 2H), 5.36 (s, 1H), 6.60 (d, *J* = 7.2 Hz, 2H), 7.00 (d, *J* = 8.8 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.20 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 2H); MS (ESI): *m/z* calcd. for C₂₁H₂₁N₂O₃ [M+H]⁺ 349.2, found: 349.3. The following compounds were similarly prepared:

Ethyl 5-[4-(2,3-dihydro-1H-perimidin-2-yl)phenoxy]pentanoate (22). Yield = 40%, mp 93.8–96.4 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.18 (t, *J* = 7.2 Hz, 3H), 1.65–1.72 (m, 4H), 2.37 (t, *J* = 7.2 Hz, 2H), 3.98 (t, *J* = 6.0 Hz, 2H), 4.06 (q, *J* = 7.2 Hz, 2H), 5.29 (s, 1H), 6.47 (d, *J* = 7.2 Hz, 2H), 6.67 (s, 2H), 6.96 (d, *J* = 8.0 Hz, 4H), 7.13 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 2H); MS (ESI): *m/z* calcd. for C₂₄H₂₇N₂O₃ [M+H]⁺ 391.2, found: 391.3.

2-[4-(2,3-Dihydro-1H-perimidin-2-yl)phenoxy]acetic acid (23). Yield = 35%, mp > 265 °C; ¹H-NMR (DMSO-*d*₆) δ: 4.33 (m, 2H), 5.27 (s, 1H), 6.47 (d, *J* = 7.6 Hz, 2H), 6.66 (s, 2H), 6.89 (d, *J* = 8.4 Hz, 2H), 6.97 (d, *J* = 8.0 Hz, 2H), 7.14 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.47 (d, *J* = 8.8 Hz, 2H); ¹³C-NMR (DMSO-*d*₆) δ: 66.1, 67.0, 104.2, 114.3, 115.1, 126.8, 128.2, 128.8, 135.0, 143.3, 152.2, 158.8, 160.9; MS (ESI): *m/z* calcd. for C₁₉H₁₇N₂O₃ [M+H]⁺ 321.1, found: 321.1.

5-[4-(2,3-Dihydro-1H-perimidin-2-yl)phenoxy]pentanoic acid (25). Yield = 50%, mp 165.9–167.8 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.63–1.75 (m, 4H), 2.29 (t, *J* = 7.2 Hz, 2H), 3.99 (t, *J* = 6.4 Hz, 2H), 5.32 (s, 1H), 6.52 (d, *J* = 7.2 Hz, 2H), 7.01 (m, 4H), 7.16 (t, *J* = 7.6 Hz, 2H), 7.51 (d, *J* = 8.8 Hz, 2H); MS (ESI): *m/z* calcd. for C₂₂H₂₃N₂O₃ [M+H]⁺ 363.2, found: 363.3.

3.1.5. General Procedure for the Preparation of Derivatives 26–33

A mixture of compound **8** (50 mg, 0.19 mmol), succinic acid monoethyl ester (27.7 mg, 0.19 mmol), EDCI (55.2 mg, 28.7 mmol) and DMAP (2.3 mg, 0.019 mmol) in DMF (2 mL) was stirred at 40 °C overnight. The reaction mixture was diluted with EtOAc (100 mL), washed with water (50 mL × 3), the organic layer was then processed in the usual way and chromatographed (1:1 petroleum ether/EtOAc) to yield compound **28** (30 mg, 41%), mp 165.8–170.9 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.18

(t, $J = 7.2$ Hz, 3H), 2.57–2.63 (m, 4H), 4.06 (q, $J = 7.2$ Hz, 2H), 5.29 (s, 1H), 6.48 (d, $J = 8.0$ Hz, 2H), 6.67 (s, 2H), 6.97 (d, $J = 8.0$ Hz, 2H), 7.14 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.51 (d, $J = 8.4$ Hz, 2H), 7.61 (d, $J = 8.4$ Hz, 2H); MS (ESI): m/z calcd. for $C_{23}H_{24}N_3O_3$ $[M+H]^+$ 390.2, found: 390.0.

A mixture of compound **28** (30 mg, 0.077 mmol) and $LiOH \cdot H_2O$ (9.7 mg, 0.23 mmol) in THF (2 mL) and H_2O (2 mL) was stirred at room temperature for 3 h. After removal of THF, the water layer was washed with EtOAc, acidified with HCl (1 M) to pH 2, filtered and dried to get compound **32** (15 mg, 54%), mp > 265 °C; 1H -NMR (DMSO- d_6) δ : 2.50–2.56 (m, 4H), 5.29 (s, 1H), 6.48 (d, $J = 8.0$ Hz, 2H), 6.67 (s, 2H), 6.97 (d, $J = 8.0$ Hz, 2H), 7.14 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.50 (d, $J = 8.4$ Hz, 2H), 7.61 (d, $J = 8.4$ Hz, 2H), 10.14 (s, 1H), 12.10 (brs, 1H); MS (ESI): m/z calcd. for $C_{21}H_{20}N_3O_3$ $[M+H]^+$ 362.2, found: 362.2. The following compounds were similarly prepared:

Ethyl 2-{{4-(2,3-dihydro-1H-perimidin-2-yl)phenyl}amino}-2-oxoacetate (26). Yield = 41%, mp 132.3–135.5 °C; 1H -NMR (DMSO- d_6) δ : 1.32 (t, $J = 7.2$ Hz, 3H), 4.31 (q, $J = 7.2$ Hz, 2H), 5.32 (s, 1H), 6.48 (d, $J = 7.6$ Hz, 2H), 6.73 (s, 2H), 6.97 (d, $J = 8.0$ Hz, 2H), 7.14 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.57 (d, $J = 8.4$ Hz, 2H), 7.77 (d, $J = 8.8$ Hz, 2H), 10.86 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 13.8, 62.4, 65.9, 104.3, 112.4, 115.2, 120.3, 126.8, 128.3, 134.4, 137.6, 138.1, 143.0, 155.6, 160.7; MS (ESI): m/z calcd. for $C_{21}H_{20}N_3O_3$ $[M+H]^+$ 362.1, found: 362.0.

Ethyl 3-{{4-(2,3-dihydro-1H-perimidin-2-yl)phenyl}amino}-3-oxopropanoate (27). Yield = 51%, mp 158.2–160.7 °C; 1H -NMR (DMSO- d_6) δ : 1.21 (t, $J = 7.2$ Hz, 3H), 3.47 (s, 2H), 4.12 (q, $J = 7.2$ Hz, 2H), 5.30 (s, 1H), 6.48 (d, $J = 8.0$ Hz, 2H), 6.69 (s, 2H), 6.97 (d, $J = 8.0$ Hz, 2H), 7.14 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.53 (d, $J = 8.0$ Hz, 2H), 7.61 (d, $J = 8.4$ Hz, 2H); MS (ESI): m/z calcd. for $C_{22}H_{22}N_3O_3$ $[M+H]^+$ 376.2, found: 375.6.

Ethyl 5-{{4-(2,3-dihydro-1H-perimidin-2-yl)phenyl}amino}-5-oxopentanoate (29). Yield = 35%, mp 139.9–142.5 °C; 1H -NMR (DMSO- d_6) δ : 1.19 (t, $J = 7.2$ Hz, 3H), 1.84 (m, 2H), 2.36 (m, 4H), 4.06 (q, $J = 7.2$ Hz, 2H), 5.29 (s, 1H), 6.48 (d, $J = 7.2$ Hz, 2H), 6.67 (s, 2H), 6.97 (d, $J = 8.0$ Hz, 2H), 7.14 (t, $J = 7.6$ Hz, 2H), 7.51 (d, $J = 8.4$ Hz, 2H), 7.62 (d, $J = 8.4$ Hz, 2H), 9.97 (s, 1H); MS (ESI): m/z calcd. for $C_{24}H_{26}N_3O_3$ $[M+H]^+$ 404.3, found: 404.1.

2-{{4-(2,3-Dihydro-1H-perimidin-2-yl)phenyl}amino}-2-oxoacetic acid (30). Yield = 35%, mp > 265 °C; 1HNMR (400MHz, DMSO- d_6) δ : 5.32 (s, 1H), 6.48 (d, $J = 7.2$ Hz, 2H), 6.73(s, 2H), 6.97 (d, $J = 8.4$ Hz, 2H), 7.14 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.56 (d, $J = 8.4$ Hz, 2H), 7.78 (d, $J = 8.4$ Hz, 2H), 10.79 (s, 1H).

3-{{4-(2,3-Dihydro-1H-perimidin-2-yl)phenyl}amino}-3-oxopropanoic acid (31). Yield = 52%, mp 221.3–223.4 °C; 1H -NMR (DMSO- d_6) δ : 3.37 (s, 2H), 5.32 (s, 1H), 6.51 (d, $J = 7.2$ Hz, 2H), 7.00 (d, $J = 8.0$ Hz, 2H), 7.15 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.54 (d, $J = 8.4$ Hz, 2H), 7.62 (d, $J = 8.4$ Hz, 2H), 10.24 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 43.9, 66.3, 105.1, 112.7, 115.9, 118.4, 118.7, 126.8, 128.5, 134.3, 139.3, 142.3, 164.6, 169.2; MS (ESI): m/z calcd. for $C_{20}H_{18}N_3O_3$ $[M+H]^+$ 348.1, found: 348.0.

5-{{4-(2,3-Dihydro-1H-perimidin-2-yl)phenyl}amino}-5-oxopentanoic acid (33). Yield = 56%, mp > 265 °C; 1H -NMR (DMSO- d_6) δ : 1.80 (m, 2H), 2.26–2.45 (m, 4H), 5.29 (s, 1H), 6.48 (d, $J = 7.2$ Hz, 2H), 6.97

(d, $J = 8.0$ Hz, 2H), 7.14 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.50 (d, $J = 8.8$ Hz, 2H), 7.62 (d, $J = 8.8$ Hz, 2H), 10.00 (s, 1H); MS (ESI): m/z calcd. for $C_{22}H_{22}N_3O_3$ $[M+H]^+$ 376.2, found: 376.1.

3.1.6. Procedure for the Preparation of Compound 34 and 35

A mixture of compound **8** (100 mg, 0.38 mmol), 3-*tert*-butoxycarbonylaminopropionic acid (72 mg, 0.38 mmol), EDCI (110 mg, 57 mmol) and DMAP (4.6 mg, 0.038 mmol) in DMF (4 mL) was stirred at 40 °C overnight. The reaction mixture was diluted with EtOAc (100 mL), washed with water (50 mL \times 3), the organic layer was then processed in the usual way and chromatographed (2:1 petroleum ether/EtOAc) to yield compound **34** (72 mg, 43%), mp 111.1–113.7 °C; $^1\text{H-NMR}$ (DMSO- d_6) δ : 1.38 (s, 9H), 2.48 (m, 2H), 3.21 (m, 2H), 5.29 (s, 1H), 6.48 (d, $J = 7.2$ Hz, 2H), 6.68 (s, 2H), 6.90 (m, 1H), 6.97 (d, $J = 8.0$ Hz, 2H), 7.14 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.51 (d, $J = 8.4$ Hz, 2H), 7.62 (d, $J = 8.4$ Hz, 2H), 10.01 (s, 1H); MS (ESI): m/z calcd. for $C_{25}H_{29}N_4O_3$ $[M+H]^+$ 433.2, found: 433.1.

Trifluoroacetic acid (1 mL) was added slowly to the solution of compound **34** (50 mg, 0.12 mmol) in DCM (5 mL) at 0 °C. After stirred at room temperature for 5 h, the solution was concentrated to yield compound **35** (26 mg, 67%), mp 239.7–241.2 °C; $^1\text{H-NMR}$ (DMSO- d_6) δ : 2.72 (m, 2H), 3.12 (m, 2H), 5.31 (s, 1H), 6.48 (d, $J = 7.2$ Hz, 2H), 6.69 (s, 2H), 6.97 (d, $J = 7.6$ Hz, 2H), 7.14 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.53 (d, $J = 8.4$ Hz, 2H), 7.63 (d, $J = 8.8$ Hz, 2H), 7.80 (brs, 2H), 10.25 (s, 1H); MS (ESI): m/z calcd. for $C_{20}H_{21}N_4O$ $[M+H]^+$ 333.2, found: 333.0.

3.1.7. Procedure for the Preparation of Compounds 40–49

SOCl_2 (28.5 mL, 0.24 mol) was added slowly to a stirred solution of compound **36** (10 g, 59.9 mmol) in methanol (100 mL) at 0 °C. After the addition, the solution was stirred at 80 °C for 4 h and then concentrated via rotary evaporator to get compound **37** (11.4 g, 98%). NaBH_4 (2.4 g, 64 mmol) was added dropwise to the mixture of compound **37** (5 g, 25.6 mmol) and CaCl_2 (11.4 g, 102.6 mmol) in THF (25 mL)/EtOH (25 mL) at 0 °C. After completion, the reaction was quenched with water. The aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way and chromatographed (1:1 petroleum ether/EtOAc) to yield compound **38** (2.5 g, 58%). Dess-Martin periodinane (3.0 g, 7.2 mmol) was added slowly to the mixture of compound **38** (1.0 g, 6.0 mmol) in DCM (10 mL). The resulting mixture continued to stir at room temperature overnight. The reaction was quenched with water. The aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way and chromatographed (3:1 petroleum ether/EtOAc) to yield compound **39** (0.85 g, 86%). To a stirred solution of compound **39** (0.3 g, 1.82 mmol) in methanol (5 mL) was added a solution of naphthalene-1,8-diamine (0.24 g, 1.52 mmol) in methanol (5 mL). Then $\text{Zn}(\text{OAc})_2$ (0.028 g, 0.128 mmol) was added and the mixture was stirred at room temperature for 16 h. The reaction mixture was filtered, the filter cake was washed with methanol, dried to get compound **40** (125 mg, 27%), mp 92.3–94.8 °C. $^1\text{H-NMR}$ (DMSO- d_6) δ : 3.88 (s, 3H), 5.50 (s, 1H), 6.54 (d, $J = 7.6$ Hz, 2H), 6.98 (d, $J = 8.0$ Hz, 2H), 7.08 (s, 2H), 7.16 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.67 (d, $J = 8.0$ Hz, 1H), 8.31 (dd, $J = 2.0$ Hz, 8.0 Hz, 1H), 9.08 (d, $J = 2.0$ Hz, 1H); MS (ESI): m/z calcd. for $C_{18}H_{16}N_3O_2$ $[M+H]^+$ 306.1, found: 306.2.

$\text{LiOH}\cdot\text{H}_2\text{O}$ (21.6 mg, 0.49 mmol) was added to a solution of compound **40** (50 mg, 0.16 mmol) in THF (2 mL) and H_2O (1 mL), then the mixture was stirred at room temperature for 3 h. After removal

of the THF, the water layer was washed with EtOAc, acidified with HCl (1 M) to pH 2, filtered and dried to yield compound **41** (20 mg, 43%), mp 100.7–101.3 °C. ¹H-NMR (DMSO-*d*₆) δ: 5.50 (s, 1H), 6.55 (d, *J* = 7.2 Hz, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 7.16 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.66 (d, *J* = 8.0 Hz, 1H), 8.29 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H), 9.07 (d, *J* = 1.2 Hz, 1H); ¹³C-NMR (DMSO-*d*₆) δ: 66.2, 104.9, 112.3, 115.6, 121.0, 126.2, 127.0, 134.2, 138.1, 141.3, 149.4, 164.8, 165.9; MS (ESI): *m/z* calcd. for C₁₇H₁₄N₃O₂ [M+H]⁺ 292.1, found: 292.5.

To a stirred solution of compound **41** (500 mg, 1.72 mmol) in DMF (10 mL) was added methyl glycinate (230 mg, 2.6 mmol), followed by EDCI (500 mg, 2.5 mmol) and DMAP (21 mg, 0.17 mmol). The mixture was stirred at 40 °C overnight. The reaction was diluted with EtOAc (100 mL), washed with water (200 mL × 3). The combined organic phases were then processed in the usual way and chromatographed (2:1 petroleum ether/EtOAc) to yield compound **42** (249 mg, 40%), mp 183.6–184.7 °C. ¹H-NMR (DMSO-*d*₆) δ: 3.66 (s, 3H), 4.04 (d, *J* = 6.0 Hz, 2H), 5.49 (s, 1H), 6.55 (d, *J* = 7.2 Hz, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 7.04 (s, 2H), 7.16 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 1H), 8.21 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H), 9.01 (d, *J* = 1.6 Hz, 1H), 9.19 (t, *J* = 5.6 Hz, 1H); MS (ESI): *m/z* calcd. for C₂₀H₁₉N₄O₃ [M+H]⁺ 363.1, found: 363.3.

LiOH·H₂O (52 mg, 1.2 mmol) was added to a solution of compound **42** (150 mg, 0.41 mmol) in THF (2 mL)/H₂O (2 mL). The reaction was stirred at room temperature for 3 h. After removal of THF, the water layer was washed with EtOAc, acidified with HCl (1 M) to pH = 2, filtered and dried to get compound **46** (60 mg, 42%), mp 103.5–107.3 °C. ¹H-NMR (DMSO-*d*₆) δ: 3.95 (d, *J* = 6.0 Hz, 2H), 5.48 (s, 1H), 6.55 (d, *J* = 7.6 Hz, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 7.04 (s, 2H), 7.16 (dd, *J* = 7.6 Hz, 8.4 Hz, 2H), 7.65 (d, *J* = 8.4 Hz, 1H), 8.22 (dd, *J* = 2.0 Hz, 8.4 Hz, 1H), 9.01 (d, *J* = 1.6 Hz, 1H), 9.08 (t, *J* = 6.0 Hz, 1H), 12.68 (brs, 1H); ¹³C-NMR (DMSO-*d*₆) δ: 41.2, 66.4, 104.6, 112.3, 115.4, 120.6, 127.0, 128.8, 134.2, 135.8, 141.7, 147.7, 163.7, 164.9, 171.0; MS (ESI): *m/z* calcd. for C₁₉H₁₇N₄O₃ [M+H]⁺ 349.1, found: 349.3. The following compounds were similarly prepared:

Methyl 4-[6-(2,3-dihydro-1H-perimidin-2-yl)nicotinamido]butanoate (43). Yield = 52%, mp 100.1–105.4 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.78 (m, 2H), 2.37 (t, *J* = 7.2 Hz, 2H), 3.28 (m, 2H), 3.57 (s, 3H), 5.47 (s, 1H), 6.54 (d, *J* = 7.2 Hz, 2H), 6.97 (d, *J* = 8.0 Hz, 2H), 7.03 (s, 2H), 7.15 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.62 (d, *J* = 8.0 Hz, 1H), 8.17 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H), 8.67 (t, *J* = 5.2 Hz, 1H), 8.97 (d, *J* = 1.6 Hz, 1H); MS (ESI): *m/z* calcd. for C₂₂H₂₃N₄O₃ [M+H]⁺ 391.2, found: 391.4.

Ethyl 5-[6-(2,3-dihydro-1H-perimidin-2-yl)nicotinamido]pentanoate (44). Yield = 60%, mp 109.4–113.5 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.16 (t, *J* = 6.8 Hz, 3H), 1.54 (m, 4H), 2.32 (m, 2H), 3.26 (m, 2H), 4.03 (q, *J* = 6.8 Hz, 2H), 5.47 (s, 1H), 6.54 (d, *J* = 7.2 Hz, 2H), 6.97 (d, *J* = 8.0 Hz, 2H), 7.03 (s, 2H), 7.15 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 1H), 8.16 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H), 8.65 (t, *J* = 5.6 Hz, 1H), 8.96 (d, *J* = 1.6 Hz, 1H); MS (ESI): *m/z* calcd. for C₂₄H₂₇N₄O₃ [M+H]⁺ 419.2, found: 419.1.

Ethyl 6-[6-(2,3-dihydro-1H-perimidin-2-yl)nicotinamido]hexanoate (45). Yield = 44%, mp 119.3–123.8 °C; ¹H-NMR (DMSO-*d*₆) δ: 1.15 (t, *J* = 7.2 Hz, 3H), 1.23–1.34 (m, 2H), 1.49–1.56 (m, 4H), 2.26–2.30 (m, 2H), 3.22–3.27 (m, 2H), 4.02 (q, *J* = 7.2 Hz, 2H), 5.47 (s, 1H), 6.45 (d, *J* = 7.2 Hz, 2H), 6.97 (d, *J* = 8.0 Hz, 2H), 7.03 (s, 2H), 7.15 (dd, *J* = 7.6 Hz, 8.0 Hz, 2H), 7.61 (d, *J* = 8.0 Hz, 1H),

8.16 (d, $J = 2.0$ Hz, 8.0 Hz, 1H), 8.62 (t, $J = 5.6$ Hz, 1H), 8.96 (d, $J = 1.6$ Hz, 1H); MS (ESI): m/z calcd. for $C_{25}H_{29}N_4O_3$ $[M+H]^+$ 433.2, found: 433.2.

4-[6-(2,3-Dihydro-1H-perimidin-2-yl)nicotinamido]butanoic acid (**47**). Yield = 50%, mp 95.3–99.0 °C; 1H -NMR (DMSO- d_6) δ : 1.72 (m, 2H), 2.21 (t, $J = 6.8$ Hz, 2H), 3.26 (m, 2H), 5.47 (s, 1H), 6.54 (d, $J = 7.2$ Hz, 2H), 6.97 (d, $J = 8.4$ Hz, 2H), 7.02 (s, 2H), 7.15 (t, $J = 7.6$ Hz, 2H), 7.61 (d, $J = 8.4$ Hz, 1H), 8.19 (dd, $J = 1.6$ Hz, 8.0 Hz, 1H), 8.99 (s, 1H), 9.33 (brs, 1H); ^{13}C -NMR (DMSO- d_6) δ : 24.6, 32.6, 39.2, 66.3, 104.6, 112.3, 115.4, 120.4, 126.9, 129.5, 134.2, 135.7, 141.7, 147.6, 163.3, 164.4, 175.0; MS (ESI): m/z calcd. for $C_{21}H_{21}N_4O_3$ $[M+H]^+$ 377.2, found: 377.1.

5-[6-(2,3-Dihydro-1H-perimidin-2-yl)nicotinamido]pentanoic acid (**48**). Yield = 46%, mp 117.1–120.3 °C; 1H -NMR (DMSO- d_6) δ : 1.54 (m, 4H), 2.24 (m, 2H), 3.25 (m, 2H), 5.47 (s, 1H), 6.54 (d, $J = 7.2$ Hz, 2H), 6.97 (d, $J = 8.0$ Hz, 2H), 7.02 (s, 2H), 7.15 (t, $J = 7.6$ Hz, 2H), 7.62 (d, $J = 8.0$ Hz, 1H), 8.17 (d, $J = 8.0$ Hz, 1H), 8.65 (t, $J = 5.2$ Hz, 1H), 8.97 (s, 1H), 12.03 (brs, 1H); ^{13}C -NMR (DMSO- d_6) δ : 22.1, 28.5, 33.4, 39.0, 66.3, 104.7, 112.4, 115.5, 120.6, 127.0, 129.5, 134.3, 135.8, 141.7, 147.6, 163.4, 164.6, 174.5; MS (ESI): m/z calcd. for $C_{22}H_{23}N_4O_3$ $[M+H]^+$ 391.2, found: 391.4.

6-[6-(2,3-Dihydro-1H-perimidin-2-yl)nicotinamido]hexanoic acid (**49**). Yield = 48%, mp 86.7–88.4 °C; 1H -NMR (DMSO- d_6) δ : 1.23–1.34 (m, 2H), 1.48–1.57 (m, 4H), 2.20 (t, $J = 7.2$ Hz, 2H), 3.24 (m, 2H), 5.48 (s, 1H), 6.55 (d, $J = 7.2$ Hz, 2H), 6.97 (d, $J = 8.0$ Hz, 2H), 7.15 (dd, $J = 7.6$ Hz, 8.0 Hz, 2H), 7.62 (d, $J = 8.0$ Hz, 1H), 8.17 (dd, $J = 2.0$ Hz, 8.0 Hz, 1H), 8.64 (t, $J = 5.6$ Hz, 1H), 8.96 (d, $J = 1.6$ Hz, 1H); ^{13}C -NMR (DMSO- d_6) δ : 24.3, 26.1, 28.7, 33.7, 39.2, 66.3, 104.8, 112.2, 115.6, 120.3, 127.0, 128.6, 134.9, 135.9, 141.6, 147.8, 163.4, 164.5, 174.5; MS (ESI): m/z calcd. for $C_{23}H_{25}N_4O_3$ $[M+H]^+$ 405.2, found: 405.3.

3.2. PTP1B and Related PTPs Biological Assay

A colorimetric assay to measure inhibition against PTP1B and TCPTP was performed in 96-well plates. Briefly, the tested compounds were solubilized in DMSO and serially diluted into concentrations for the inhibitory test. The assays were carried out in a final volume of 100 μ L containing 50 mmol/L MOPS, pH 6.5, 2 mmol/L pNPP, 30 nmol/L GST-PTP1B or GST-TCPTP, and 2% DMSO, and the catalysis of pNPP was continuously monitored on a SpectraMax 340 microplate reader at 405 nm for 3 min at 30 °C. The IC_{50} value was calculated from the nonlinear curve fitting of the percent inhibition [inhibition (%)] vs. the inhibitor concentration using the Equation (1):

$$\text{Inhibition (\%)} = 100/[1 + (IC_{50}/[I])^k] \quad (1)$$

where k is the Hill coefficient. To study the inhibition on the other PTPase family members, SHP1, SHP2 and LAR were prepared and assays were performed according to procedures described previously [24,25]. Briefly, the enzymatic activity of the SHP1, SHP2 and LAR were determined at 30 °C by monitoring the dephosphorylation of substrate 3-o-methylfluorescein phosphate (OMFP), product was then detected at a 485 nm excitation wavelength and 530 nm emission wavelength by the EnVision multilabe plate reader (Perkin-Elmer Life Sciences, Boston, MA, USA). The assays were carried out in a final volume of 50 μ L containing 50 mmol/L MOPS, pH 6.8, 10 μ mol/L OMFP, 20 nmol/L

recombinant enzyme, 2 mmol/L dithiothreitol, 1 mmol/L EDTA, and 2% DMSO. The initial rate of the dephosphorylation was presented by the early linear region of the enzymatic reaction kinetic curve, the inhibitory activity of the compound was continuously monitored.

3.3. Characterization of the Inhibitor on Enzyme Kinetics [25]

In the fast-binding inhibition experiment, PTP1B were preincubated with compounds (2% DMSO) on the ice for different times, and then add 10 μ L mixture of enzyme and compounds to 90 μ L assay system. To characterize the inhibitor of PTP1B, the assay was carried out in a 100 μ L system containing 50 mmol/L MOPS, pH 6.5, 14 nmol/L PTP1B, *p*NPP in 2-fold dilution from 80 mmol/L, and different concentrations of the inhibitor. In the presence of the competitive inhibitor, the Michaelis-Menten equation is described as Equation (2):

$$1/v = [K_m/(V_{max}[S])] \times (1 + [I]/K_i) + 1/V_{max} \quad (2)$$

where K_m is the Michaelis constant, v is the initial rate, V_{max} is the maximum rate, and $[S]$ is the substrate concentration. The K_i value was obtained by the linear replot of apparent K_m/V_{max} (slope) from the primary reciprocal plot *versus* the inhibitor concentration $[I]$ according to the equation $K_m/V_{max} = 1 + [I]/K_i$.

3.4. Cellular Activity of Compound 46

CHO/hIR cells were cultured in F12 nutrient medium including 10% (V/V) FBS, 100 units/mL penicillin and 100 μ g/mL streptomycin with 5% CO₂ at 37 °C. Cells were serum free starved for 2 h, and then treated with compounds for 3 h, followed with insulin (10 nM, Eli Lilly) for 10 min before harvested. Cells were rinsed twice with precooled 1 \times PBS and then lysed with 1 \times SDS loading buffer. Samples were heated at 100 °C for 15 min before electrophoresed with 8% SDS-polyarylamide gel under 80 to 120 volt voltage, and then transferred to nitrocellulose (NC) membranes. NC membranes were blocked for 2 h with 5% BSA (W/V) dissolved in TBST. The membranes incubated with primary antibodies overnight at 4 °C and secondary antibodies for 1 h at room temperature. The primary antibody p-Tyr (PY20) used was from Santa Cruz (Dallas, CA, USA) and β -actin from Sigma (St. Louis, MO, USA), secondary antibody was from Jackson Immuno Research (Philadelphia, PA, USA).

4. Conclusions

In conclusion, a series of 1*H*-2,3-dihydroperimidine derivatives were synthesized and identified as PTP1B inhibitors with IC₅₀ in the micromolar range. After performing systematic SAR studies, we identified two compounds with IC₅₀ values less than 1 μ M. Among these, the representative compounds had no visible activities against receptor-like transmembrane LAR. Compound 46 possessed about 9-fold selectivity for PTP1B over SHP-1 and SHP-2, respectively. More importantly, compound 46 exhibited 3.48-fold selectivity for PTP1B over TCPTP, and cellular activity for protection of phosphorylation of IR. These results provide a possible opportunity for the development of novel PTP1B inhibitors with promising cell permeability, bioavailability, and improved pharmacological properties.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Hunter, T. Tyrosine phosphorylation: Thirty years and counting. *Curr. Opin. Cell Biol.* **2009**, *21*, 140–146.
2. Zhang, Z.Y. Protein tyrosine phosphatases: Prospects for therapeutics. *Curr. Opin. Chem. Biol.* **2001**, *5*, 416–423.
3. Julien, S.G.; Dube, N.; Hardy, S.; Tremblay, M.L. Inside the human cancer tyrosine phosphatome. *Nat. Rev. Cancer* **2011**, *11*, 35–49.
4. Zhang, J.; Yang, P.L.; Gray, N.S. Targeting cancer with small molecule kinase inhibitors. *Nat. Rev. Cancer* **2009**, *9*, 28–39.
5. Knight, Z.A.; Lin, H.; Shokat, K.M. Targeting the cancer kinome through polypharmacology. *Nat. Rev. Cancer* **2010**, *10*, 130–137.
6. Kim, S.J.; Ryu, S.E. Structure and catalytic mechanism of human protein tyrosine phosphatome. *BMB Rep.* **2012**, *45*, 693–699.
7. Tonks, N.K. Protein tyrosine phosphatases—from housekeeping enzymes to master regulators of signal transduction. *FEBS J.* **2013**, *280*, 346–378.
8. Janne, P.A.; Gray, N.; Settleman, J. Factors underlying sensitivity of cancers to small-molecule kinase inhibitors. *Nat. Rev. Drug. Discov.* **2009**, *8*, 709–723.
9. He, Y.; Liu, S.; Menon, A.; Stanford, S.; Oppong, E.; Gunawan, A.M.; Wu, L.; Wu, D.J.; Barrios, A.M.; Bottini, N.; *et al.* A potent and selective small-molecule inhibitor for the lymphoid-specific tyrosine phosphatase (LYP), a target associated with autoimmune diseases. *J. Med. Chem.* **2013**, *56*, 4990–5008.
10. Moller, D.E. New drug targets for type 2 diabetes and the metabolic syndrome. *Nature* **2001**, *414*, 821–827.
11. Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A.L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C.C.; *et al.* Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* **1999**, *283*, 1544–1548.
12. Klamann, L.D.; Boss, O.; Peroni, O.D.; Kim, J.K.; Martino, J.L.; Zabolotny, J.M.; Moghal, N.; Lubkin, M.; Kim, Y.B.; Sharpe, A.H.; *et al.* Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol. Cell Biol.* **2000**, *20*, 5479–5489.
13. Lessard, L.; Stuble, M.; Tremblay, M.L. The two faces of PTP1B in cancer. *Biochim. Biophys. Acta* **2010**, *1804*, 613–619.

14. Yip, S.C.; Saha, S.; Chernoff, J. PTP1B: A double agent in metabolism and oncogenesis. *Trends Biochem. Sci.* **2010**, *35*, 442–449.
15. He, R.; Zeng, L.F.; He, Y.; Zhang, S.; Zhang, Z.Y. Small molecule tools for functional interrogation of protein tyrosine phosphatases. *FEBS J.* **2013**, *280*, 731–750.
16. Sobhia, M.E.; Paul, S.; Shinde, R.; Potluri, M.; Gundam, V.; Kaur, A.; Haokip, T. Protein tyrosine phosphatase inhibitors: A patent review (2002–2011). *Expert Opin. Ther. Pat.* **2012**, *22*, 125–153.
17. Barr, A.J. Protein tyrosine phosphatases as drug targets: Strategies and challenges of inhibitor development. *Future Med. Chem.* **2010**, *2*, 1563–1576.
18. Erbe, D.V.; Wang, S.; Zhang, Y.L.; Harding, K.; Kung, L.; Tam, M.; Stolz, L.; Xing, Y.; Furey, S.; Qadri, A.; *et al.* Ertiprotafib improves glycemic control and lowers lipids via multiple mechanisms. *Mol. Pharmacol.* **2005**, *67*, 69–77.
19. Lantz, K.A.; Hart, S.G.; Planey, S.L.; Roitman, M.F.; Ruiz-White, I.A.; Wolfe, H.R.; McLane, M.P. Inhibition of PTP1B by trodusquemine (MSI-1436) causes fat-specific weight loss in diet-induced obese mice. *Obesity* **2010**, *18*, 1516–1523.
20. Dai, H.L.; Gao, L.X.; Yang, Y.; Li, J.Y.; Cheng, J.G.; Li, J.; Wen, R.; Peng, Y.Q.; Zheng, J.B. Discovery of di-indolinone as a novel scaffold for protein tyrosine phosphatase 1B inhibitors. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7440–7443.
21. Iversen, L.F.; Moller, K.B.; Pedersen, A.K.; Peters, G.H.; Petersen, A.S.; Andersen, H.S.; Branner, S.; Mortensen, S.B.; Moller, N.P. Structure determination of T cell protein-tyrosine phosphatase. *J. Biol. Chem.* **2002**, *277*, 19982–19990.
22. Belmonte, M.M.; Escudero-Adán, E.C.; Benet-Buchholz, J.; Haak, R.M.; Kleij, A.W. Facile synthesis of substituted Mono-, Di-, Tri- and Tetra-2-aryl-2,3-dihydro-1H-perimidines. *Eur. J. Org. Chem.* **2010**, 4823–4831.
23. Chong, H.S.; Torti, S.V.; Ma, R.; Torti, F.M.; Brechbiel, M.W. Synthesis and potent antitumor activities of novel 1,3,5-*cis,cis*-triaminocyclohexane *N*-pyridyl derivatives. *J. Med. Chem.* **2004**, *47*, 5230–5234.
24. Shi, L.; Yu, H.P.; Zhou, Y.Y.; Du, J.Q.; Shen, Q.; Li, J.Y.; Li, J. Discovery of a novel competitive inhibitor of PTP1B by high-throughput screening. *Acta Pharmacol. Sin.* **2008**, *29*, 278–284.
25. Zhang, W.; Hong, D.; Zhou, Y.; Zhang, Y.; Shen, Q.; Li, J.Y.; Hu, L.H.; Li, J. Ursolic acid and its derivative inhibit protein tyrosine phosphatase 1B, enhancing insulin receptor phosphorylation and stimulating glucose uptake. *Biochim. Biophys. Acta* **2006**, *1760*, 1505–1512.
26. McGovern, S.L.; Helfand, B.T.; Feng, B.; Shoichet, B.K. A specific mechanism of nonspecific inhibition. *J. Med. Chem.* **2003**, *46*, 4265–4272.

Sample Availability: Samples of the compounds **1–6**, **8–19**, **21–35** and **40–49** are available from the authors.

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