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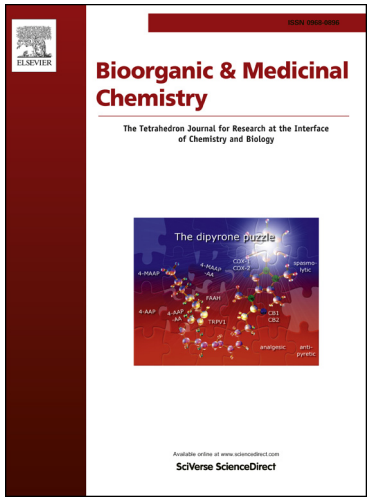

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Novel, Potent, Selective and Cellular Active ABC Type PTP1B Inhibitors Containing (Methanesulfonyl-phenyl-amino)-acetic acid methyl ester Phosphotyrosine Mimetic

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Abstract:

Protein tyrosine phosphatase1B (PTP1B) which plays an important role in the negative regulation of insulin and leptin pathway has emerged as a novel promising therapeutic target for the treatment of type 2 diabetes mellitus and obesity. Upon careful study, a series of novel scaffold and simple synthesis method inhibitors were discovered based on the analysis of X-ray crystal structures of PTP1B/inhibitor complexes and docking simulations. Among them, compound **P7** exhibited high inhibitory activity (IC_{50} = 222 nM) with moderate selectivity (8-fold) over T-cell PTPase (TCPTP) through interacting with the A, B and C binding sites of PTP1B enzyme. Further studies on cellular activities revealed that compound **P7** could enhance insulin-mediated IRβ phosphorylation and insulin-stimulated glucose uptake.

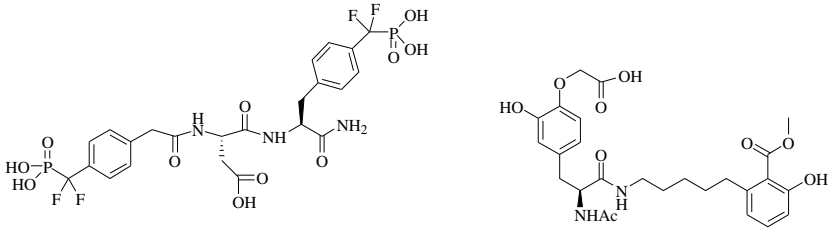
Key words

Type 2 diabetes mellitus PTP1B Inhibitor Structure–activity relationships

1. Introduction

Type 2 diabetes mellitus (T2DM) is a multi-cause metabolic diseases resulting from insulin deficiency and insulin resistance which is associated not only with the conduction disturbance of signal transduction but also with the aberration of interaction between insulin and insulin receptor (IR).[1] In insulin signaling pathway of normal individuals, insulin is combined with α subunits of IR which subsequently is activated resulting in the autophosphorylation of both subunits and IR on tyrosine residues. Following activation, IR substrate (IRS) is phosphorylated successively and then the phosphatidylinositol 3 kinase (PI3K)/AKT pathway is activated, while promotes the transcription and translation of glucose transporters (GLUTs) and guides GLUTs from the cytoplasm to the membrane to promote the uptake of glucose by cells ultimately.[2] However, PTP1B as a negative regulator for the signaling pathway of insulin and leptin dephosphorylates the phosphorylation of IR,[3,4] IRS and Janus kinase 2 (JAK2),[5-7] which is relevant to the signal transduction of leptin,[8] then the signal transduction of insulin pathway are blocked resulting in T2DM and obesity on a global scale.[9] The study results of PTP1B knockout mice have demonstrated that mice lacking the PTP1B displayed improved insulin sensitivity and resistance to obesity.[10, 11] Interestingly, gene deletion of PTP1B did not affect the normal growth and longevity in mice.[12] Therefore, PTP1B as a novel promising therapeutic target has become a hot spot of research for the treatment of T2DM and obesity.[13]

Accordingly, various higher activities of PTP1B inhibitors have been developed in the last decade.[14, 15] However, only two inhibitors can do further research into the clinical.[16-18] Poor cell permeability, low oral bioavailability and weak selectivity for the active site[19, 20] are the major reasons of the many previously disclosed PTP1B inhibitors such as compounds **1** and **2** which respectively contain the bis-anionic on difluoromethyl phosphonates (DFMP) moiety and the carboxymethylsalicyclic acids (CMS) moiety with monoacid.[21, 22]



Compound 1
K_i = 2.4 nM (PTP1B)
K_i = 24 nM (TCPTP)

Compound 2
K_i = 9.0 μM (PTP1B)
K_i =182 μM (TCPTP)

Figure 1. Structures of reported DFMP moiety (Compound **1**) and CMS moiety (Compound **2**).

Multiple positively charged sites (A, B, C, D and E) were demonstrated in the PTP1B enzyme.[23] The A site is the positively charged catalytic phosphate binding pocket with 9-Å deep (from Cys215 to Phe182) and 10-Å wide (from Tyr46 to Gln262), which contains the catalytic Cys215 in the polar phosphate binding loop (Cys215–Arg221) and hydrophobic residues (Y46, V49, F182, A217, I219, and Q262) that interact with the aryl ring of phosphotyrosine (pTyr). The B site is a noncatalytic secondary phosphate binding pocket which is larger (13×20 Å) and shallower (>4 Å) than the A site and contains several polar and hydrophobic residues. The C site is a large flat region that can accommodate large negatively charged substituents. The D site is a small pocket that shielded from solvent and adjacent to the A site, and the E site is a highly solvent exposed region located over the flap (Phe182) of the protein.[23] A comprehensive review of the reported PTP1B inhibitors proposed that the inhibitors which bind in the A site and extend into at least one additional site will improve not only the binding affinity but also the opportunity for selectivity over TCPTP.[23]

More recently, our research group has reported the discovery of N-(2, 5-diethoxy-phenyl)-methanesulfonamide based compounds as novel, potent, selective and cellular active ADC type PTP1B inhibitors which interact with the A, D and C site through fragment-docking-oriented de novel design.[24] In this paper, the discovery of (methanesulfonyl-phenyl-amino)-acetic acid methyl ester analogues as novel potent, selective and cellular active ABC type PTP1B inhibitors will be reported in details.

2. Inhibitor Design

In the A site, the structures of these PTP1B inhibitors were purposefully designed based on the analysis of X-ray crystal structures of DFMP moiety and CMS moiety bounding into the PTP1B pTyr active site (PDB code 1Q6T). A dense of hydrogen bonds were observed between DFMP moiety and Phe182, Ser216, Ala217, Tle219, Gly220, Arg221 (Fig. 2a), while the CMS moiety

formed a number of hydrogen bonds with the Lys120, Gly220 and Arg221 (Fig. 2b).

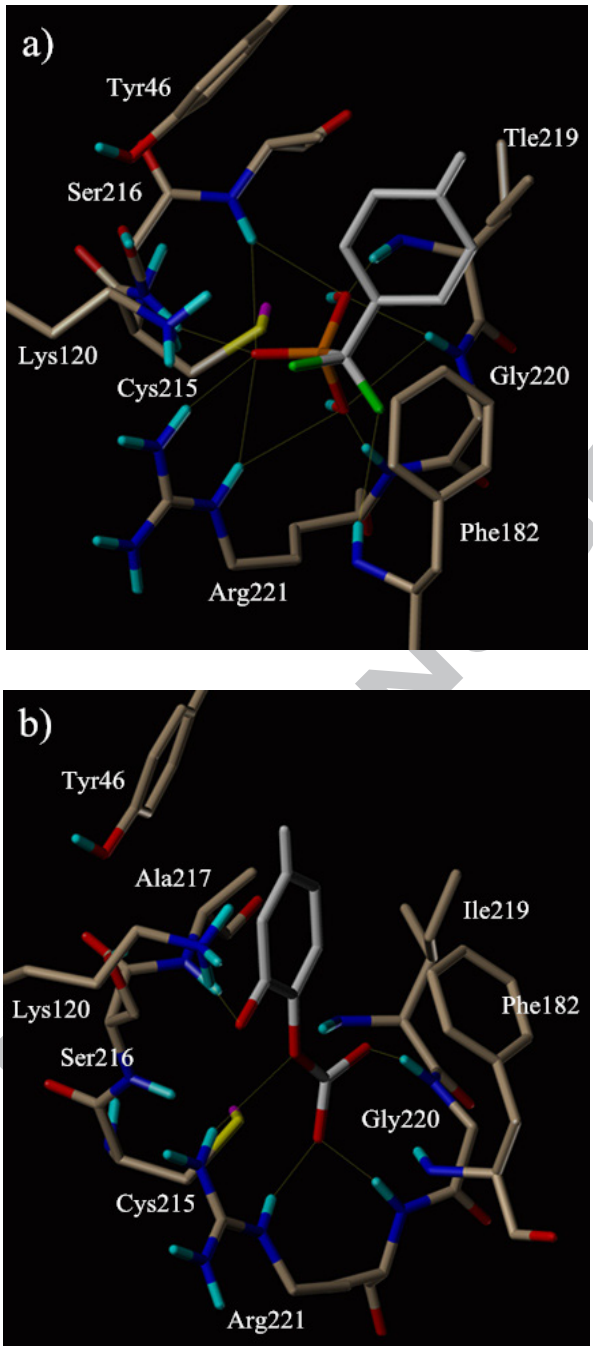


Figure 2. Binding mode of DFMP moiety and CMS moiety to the PTP1B pTyr active site derived from docking simulations showing respectively in Figure a and b. Carbon is in dark gray for PTP1B and gray for ligands, oxygen atoms are in red, nitrogen is in blue, sulfur is in yellow, fluorine is in green, phosphorus is in orange and hydrogen is in cyan. Hydrogen bonds are shown as dashed yellow lines. These images were generated using the Sybyl-x program.

In order to eliminate the charge while retaining the proton-accepting capability, the neutral methyl sulfonyl group and methyl acetate group were consolidated into one fragment to replace the

-CF_2PO_3 group on DFMP moiety and monoacid group CMS moiety in the A site. The hydrogen bond acceptors of two sulfonyl oxygens effectively mimic the oxygens of the DFMP moiety, while the ester group mimics the CMS moiety carboxyl group in the deep A site. The 3D docking model of fragment **3** to PTP1B was constructed using Surflex-dock docking module within the software Sybyl X 1.0. (PDB code 1Q6T). The two sulfonyl oxygens and the oxygens of ester group in fragment **3** form a total of six hydrogen bonds with the backbone NHs of Lys120, Ser216, Ala217 at the bottom of the active site (Fig. 3). Clearly, the fragment **3** is able to achieve most hydrogen-bonding interactions that the DFMP moiety and CMS moiety make with the active site.

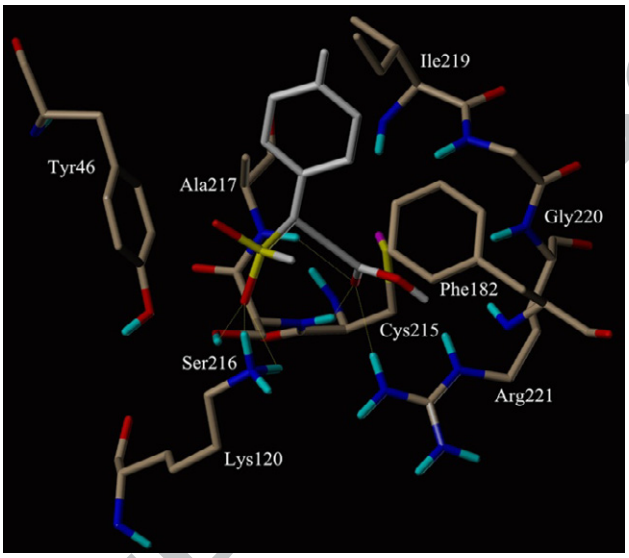


Figure 3. Binding mode of fragment **3** to the PTP1B pTyr active site. This image was generated using the Sybyl-x program.

Then the chain length was increased to the aryl substituent of fragment **3** to reach further into the B site, which may simultaneously increase the affinity and specificity.[23] Therefore, the sulfamide were introduced and attempted to interact with the corresponding residues in the B site through van der waals force, hydrophobic interaction or hydrogen-bond interaction (Fig. 4). Meanwhile, the chain extending to the C site was anticipated. So the R_2 groups were changed to interact with the C site, which probably had an impact on the inhibitory activity or selectivity (Fig. 4). Consequently, compound **P5**-**P16** with nitrogen atom at their centers were designed extending to the A, B and C sites.

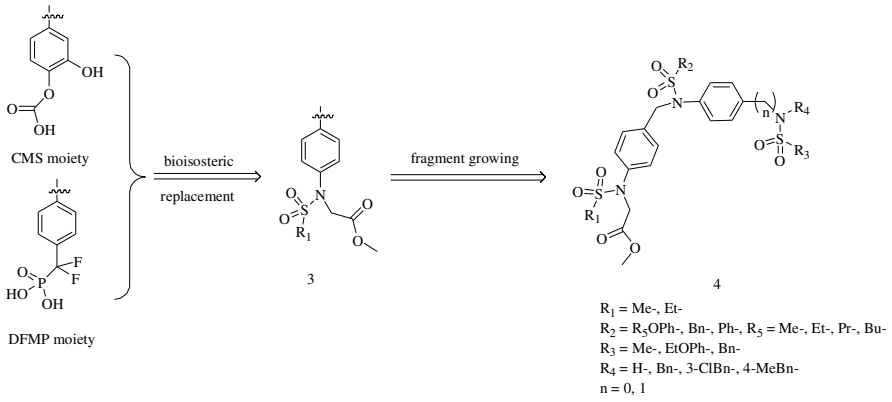
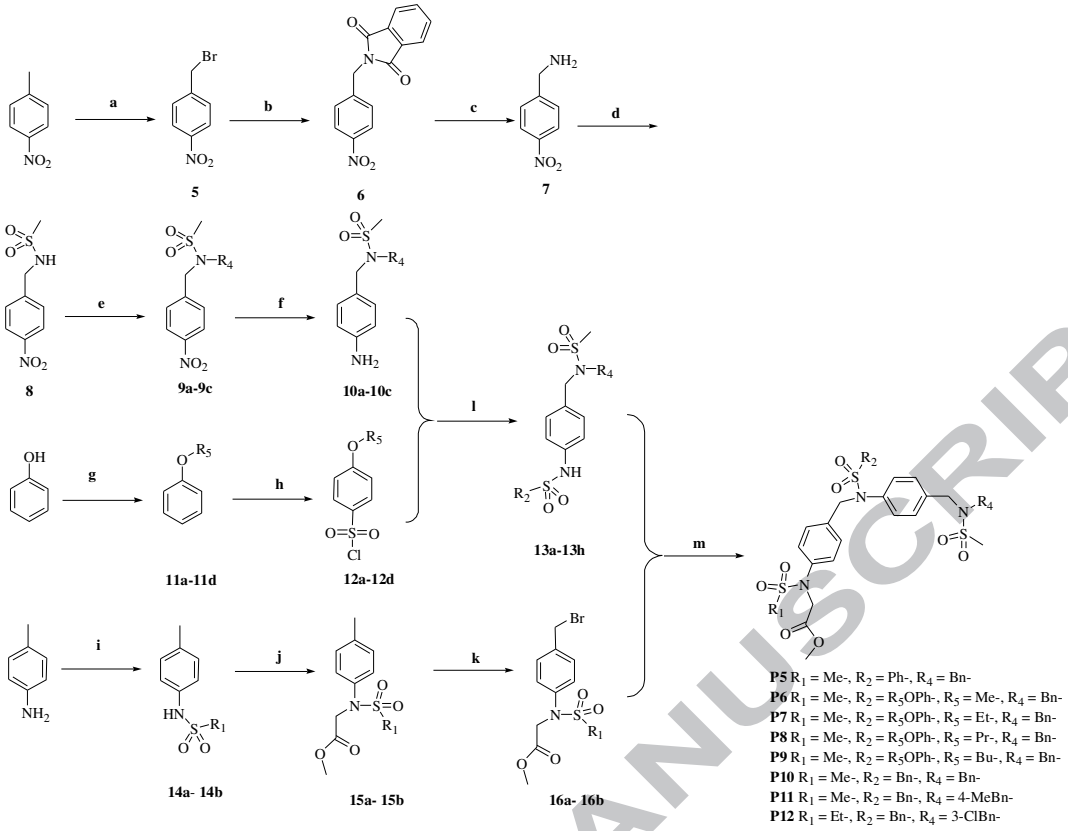


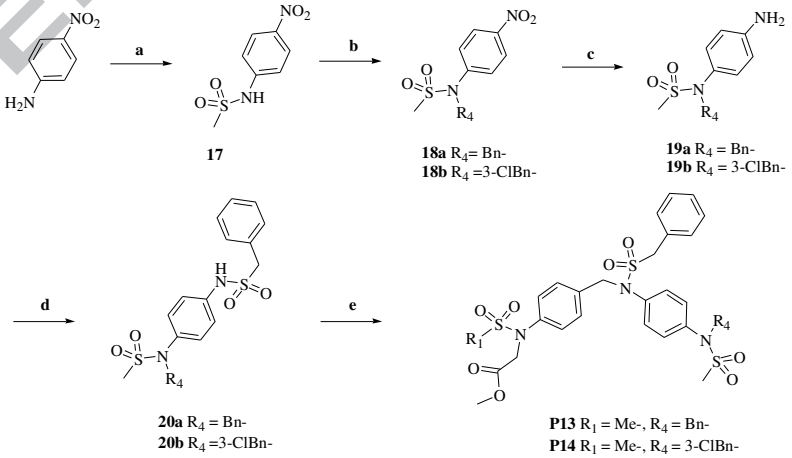
Figure 4. Fragment design and the growth of PTP1B inhibitors.

Synthesis

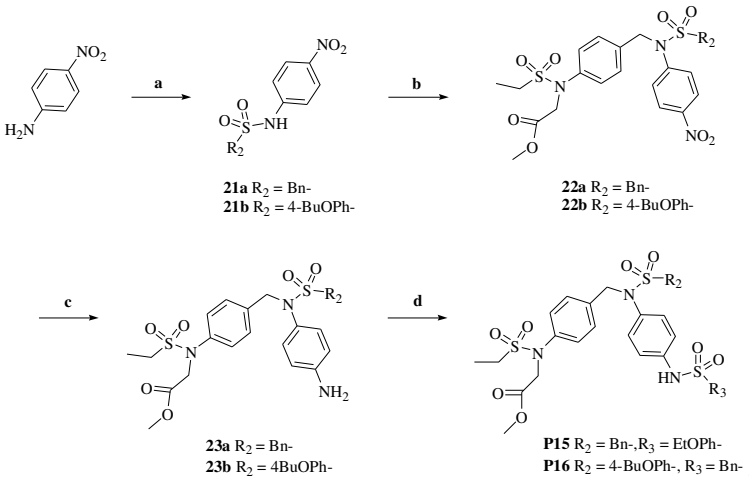
The general method for the synthesis of **P5-P12** is depicted in Scheme 1. Intermediate **5** was carried out in high yield by the bromination of commercially available 1-methyl-4-nitro-benzene with N-bromosuccinimide (NBS),[25] subsequent amination with Gabriel synthesis afforded 4-nitro-benzylamine.[26] Then intermediates **9a-9c** were effectively prepared by alkylation of **8** with α-halogenated aromatic hydrocarbons followed by sulfonylation with methanesulfonyl chloride.[27, 28] After nitro reduction, intermediates **9a-9c** were converted to **10a-10c** in good yield.[29] Subsequent sulfonylation with benzenesulfonyl chloride or benzylsulfonyl chloride or benzenesulfonyl chloride derivatives afforded intermediates **13a-13h**.[30] The benzenesulfonyl chloride derivatives (**12**) were synthesized by alkylation of phenol and of later sulphonation with chlorosulfonic acid.[31, 32] Then substitution reaction with **16a-16b** which was obtained by bromination with NBS followed by sulfonylation of 4-methylaniline with alkylsulfonyl chloride and alkylation of **14a-14b** with methyl bromoacetate separately provided compounds **P5**-**P12**. Compounds **P13**, **P14** were prepared in a similar manner followed by sulfonylation, alkylation, nitro reduction, sulfamide formation and alkylation in scheme 2. Compounds **P15**, **P16** were also carried out according to the steps of scheme 1 followed by the formation of sulfonamide, alkylation, nitro reduction and sulfonylation in scheme 3.



Scheme 1.(a) NBS, BPO, CCl_4, reflux, 75°C,10 h; (b) phthalimide potassium, TBAB, DMF, reflux, 100°C, 5 h; (c) $N_2H_4{\cdot}H_2O$, methanol, reflux, 60°C, 3 h; (d) C_5H_5N, DCM , N_2, 0°C, 20 min; (e) DMF, K_2CO_3, rt, 60-70°C, 3 h; (f) $NiCl_2{\cdot}6H_2O$; $NaBH_4$, methanol, 0°C, 30 min; (g) R_5 = Me, KOH, DMS, methanol, 0-60°C, 5 h; R_5 = Et, Pr, Bu K_2CO_3, DMF, refluxed, 60-70°C, 5 h; (h) chlorosulfonic acid, DCM, 0°C, 2 h; (i) C_5H_5N, DCM, N_2, rt, 12 h; (j) methyl bromoacetate, NaH, DMF, N_2, rt, 12 h; (k) NBS, AIBN, CCl_4, reflux, 80°C, 10 h; (l) C_5H_5N, DMAP, DCM, N_2, rt, overnight; (m) KI, K_2CO_3, DMF, rt, overnight.



Scheme 2. (a) C_5H_5N, DCM, N_2, rt, overnight; (b) DMF, K_2CO_3, rt, 3 h; (c) $NiCl_2{\cdot}6H_2O$; $NaBH_4$, methanol, 0°C, 30 min; (d) C_5H_5N, DMAP, DCM, N_2, rt, overnight; (e) KI, K_2CO_3, DMF, rt, overnight.



Scheme 3.(a) C_5H_5N, DMAP, DCM, N_2, rt, overnight; (b) KI, K_2CO_3, DMF, rt, overnight; (c) $NiCl_2{\cdot}6H_2O$, $NaBH_4$, methanol, 0°C; 30 min; (d) C_5H_5N, DCM, N_2, rt, overnight.

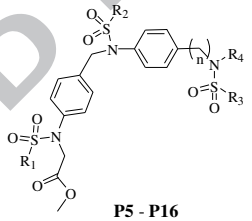
4. Results and discussion

4.1. PTP1B enzyme inhibitory activity and structure–activity relationship

All the synthesized compounds were evaluated for inhibitory intensity against PTP1B enzyme,[33, 34] and the results were shown in Table 1. According to evaluation results, we analyzed the preliminary structure–activity relationships. Compounds **P10** (n = 1) and **P12** (n = 1) showed no inhibitory activity when benzyl group and 3-chlorobenzyl were respectively introduced to R_4 and compounds **P11** (n = 1, IC_{50} = 20338 nM) was almost no inhibitory activity when R_4 was 4-methylbenzyl. Excitingly, compared to compound **P10**, the removal of methylene in compound **P13** (n = 0) led to a significant increase in inhibitory potency with IC_{50} of 492 nM. Conversely, the potency was lost when 3-chlorobenzyl was introduced at R_4 (**P14**, n = 0). Among **P10**-**P14**, compound **P13** (n=0) exhibited excellent inhibitory activity, perhaps because the relatively small volume structure (n = 0, with nothing substituent in B site) provided a preferred conformation adapted to the B site, while the volume of compounds **P10**-**P12** (n = 1) and compound **P14** (n = 0, with chloric substituent in B site) in B site were too large and difficult to adapt to the size of the pocket when R_2 were all the flexible and bulky benzyl. Compound **P15** (n = 0) exhibited the same level of inhibitory activity (IC_{50} = 470 nM) when R_1, R_3 and R_4 were ethyl-, 4-ethoxy-phenyl groups and hydrogen respectively compared with compound **P13**. However, compared to compound **P15**, 4-fold decrease of inhibitory potency was observed when R_2, R_3 and R_4 were 4-butoxy-phenyl, benzylsulfonylamino groups and hydrogen respectively in compound **P16** (n = 0, IC_{50} = 1709 nM). Interestingly, compound **P9** (n = 1, IC_{50} = 896 nM) displayed 2-fold more potent than compound

P16 although with the same substitute at R_2. That demonstrated the positive role of methylsulfonyl group in B site may be not obvious and n=1 was more suitable for B site when R_2 was 4-butoxy-phenyl group instead of benzyl group. Compound **P8** (n = 1, IC_{50} = 902 nM) displayed the same level of inhibitory activity compared with compound **P9** when the 4-propoxy-phenyl was introduced to R_2. Excitingly, the inhibitory activity was dramatically increased and displayed 4- fold more potent than compound **P8** when the 4-ethoxy-phenyl was introduced to R_2 (**P7**, n = 1, IC_{50} = 222 nM). Conversely, the introduction of 4-methoxy - phenyl (**P6**, n = 1, IC_{50} = 346 nM) or phenyl group (**P5**, n = 1, IC_{50} = 16098 nM) to the same position respectively resulted in about 1.5-fold and 72-fold decrease of inhibitory potency than compound **P7**. Hence, compounds **P5-P9** demonstrated a large volume structure (n = 1) in B site with 4-ethoxy-pheny at R_2 provided a preferred conformation adapted to the pockets of B and C. According to the shape characteristics of PTP1B pockets, compound **P5**-**P16** indicated that the shape and length of whole structure from B to C site played a very important role in inhibitory activity of compounds based on the steady binding affinity of structure in the A site. Among them, compound **P7** with the optimum configuration was a novel promising lead compound.

Table1. Inhibitory activity of compounds **P5-P16** against PTP1B [a]



P5 - P16

NO.	n	R_1	R_2	R_3	R_4	IC_{50} (nM) mean ± SEM
P5	1	Me-	Ph-	Me-	Bn-	16098 ± 3954
P6	1	Me-	4-MeOPh-	Me-	Bn-	346 ± 98
P7	1	Me-	4-EtOPh-	Me-	Bn-	222 ± 53
P8	1	Me-	4-n-PrOPh-	Me-	Bn-	902 ± 105
P9	1	Me-	4-n-BuOPh-	Me-	Bn-	896 ± 46
P10	1	Me-	Bn-	Me-	Bn-	NA [b]
P11	1	Me-	Bn-	Me-	4-MeBn-	20338 ± 724
P12	1	Et-	Bn-	Me-	3-ClBn-	NA
P13	0	Me-	Bn-	Me-	Bn-	492 ± 106

P14	0	Me-	Bn-	Me-	3-ClBn-	NA
P15	0	Et-	Bn-	EtOPh-	H	470 ± 146
P16	0	Et-	4-n-BuOPh-	Bn-	H	1709 ± 625

[a] Values represent triplicate determinations.

[b] NA: Inhibition rate <50% at 25 μM concentration.

4.2. The selectivity of compounds P6, P7, P13 and P15 over TCPTP, SHP-1, SHP-2, LAR

Subsequently, the potent active compounds **P6**, **P7**, **P13** and **P15** with IC_{50} below 1μM were selected to test against other PTPs (TCPTP, SHP-1, SHP-2 and LAR). As shown in Table 2, compound **P6** displayed no enzyme inhibitory activities against other PTPs. Similarly, no inhibition of SHP2 and LAR was observed in compound **P7** with 8-fold and 10-fold selectivity for PTP1B over TCPTP and SHP1. Compound **P13** exhibited about 20-fold PTP1B selectivity than for SHP1 and SHP2, and showed no inhibition for TCPTP and LAR, while compound **P15** showed about 2-fold decreased selectivity than compound **P7** for TCPTP, but 2-fold increased selectivity for SHP1.

Table 2. The inhibitory activity of select compounds **P6**, **P7, P13**, **P15** as PTP1B inhibitors against a panel of PTPs, IC_{50} [a]

Compound	PTP1B (nM)	TCPTP (nM)	SHP1 (nM)	SHP2 (nM)	LAR (nM)
oleanolic acid	153 6 ± 267	5460 ± 740			
Na_3VO_4			14190 ± 2630	11620 ± 2840	22310 ± 4670
P6	346 ± 98	NA[b]	NA	NA	NA
P7	222 ± 53	1860 ± 270	2310 ± 440	NA	NA
P13	492 ± 106	NA	9610 ± 1350	11640 ± 2190	NA
P15	470 ± 146	1920 ± 260	11360 ± 1870	NA	NA

[a] The pNPP assay. IC_{50} values were determined by regression analysis and expressed as means ± SD of three replications.

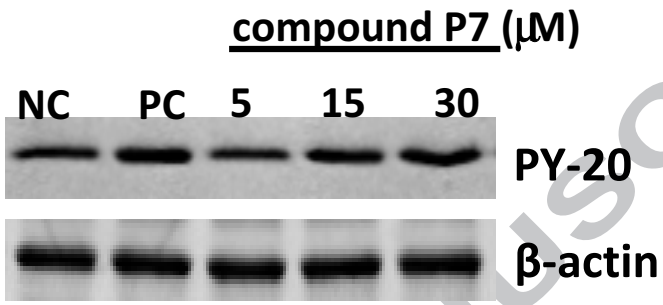
[b] NA: Inhibition rate <50% at 25 μM concentration.

4.3. The cellular activity of compound P7

Previous studies have suggested a pivotal role for the intrinsic tyrosine kinase activity of the IRβ in insulin-mediated signalling cascades.35 PTP1B participates in the dephosphorylation and inactivation of IR, thus attenuating insulin signaling.34 It has been verified that inhibition of PTP1B

by specific antisense oligonucleotide36 or inhibitors results in improvement of hyperglycaemia and insulin sensitivity in diabetic animal models.37, 38 Thus, we evaluated the effects of compound P7 on phosphorylation level of IRβ both in CHO cells transfected with hIRβ-expressing plasmid (Figure 5A) and in 3T3-L1 preadipocytes (Figure 5B). As shown in Figures 5A and 5B, compound P7 enhanced insulin-mediated IRβ phosphorylation at concentrations of 15 μM and 30 μM.

5A



5B

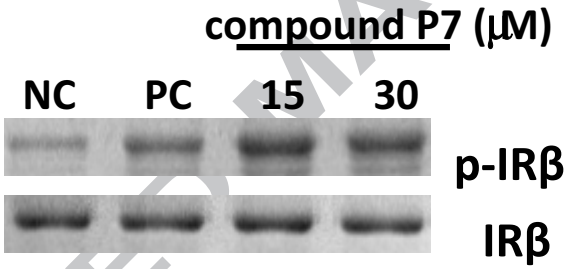
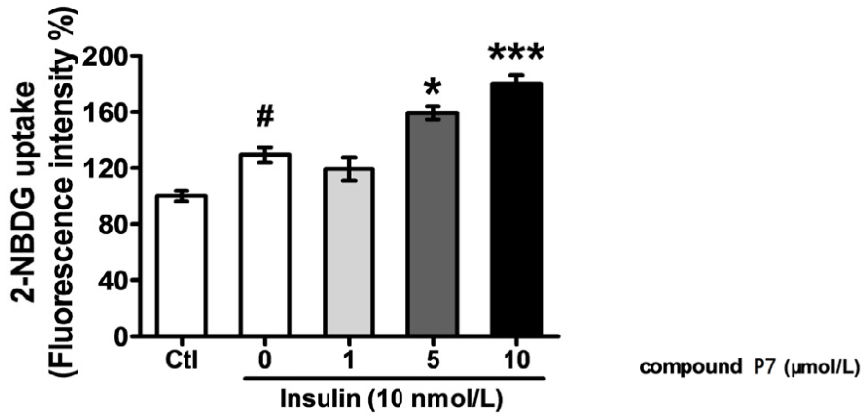


Figure 5A and 5B. Effect of compound **P7** on tyrosine phosphorylation of insulin receptor β (IRβ) in CHO cells transfected with hIRβ-expressing plasmid (5A) or in 3T3-L1 cells (5B). NC, negative control; PC, positive control.

It has been suggested that PTP1B inhibition results in a marked improvement in insulin sensitivity and glucose tolerance,[38] we investigated whether the PTP1B inhibition exerted by **P7** also results in enhanced insulin-stimulated glucose uptake. As shown in Figure 6A, insulin-stimulated glucose uptake was significantly increased in L6 myotubes treated with **P7**, and this increase was 23.3% and 29.3% at 5 and 10 μM, respectively. Furthermore, **P7** also enhanced insulin-stimulated glucose uptake in human skeletal muscle cells (HSkMC) in a dose-dependent manner (Figure 6B).

6A



6B

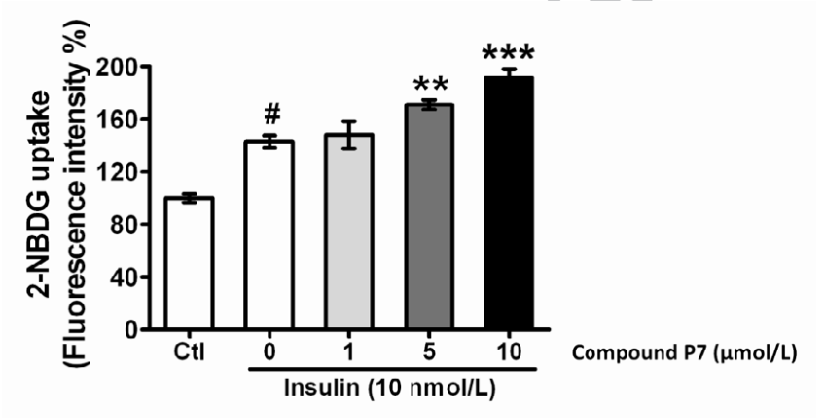


Figure 6A and 6B. Effects of compound **P7** on insulin-stimulated glucose uptake in L6 myotubes (6A) or in human skeletal muscle cells (HSkMC). 6**A**, L6 cells or 6**B**, HSkMC were serum starved for 2 h and then incubated with varying concentration of compound **P7** (0, 1, 5 and 10 µM). After 4 h, insulin (10 nM) stimulated glucose uptake was evaluated using 2-NBDG as described in Methods. Values are presented as mean ± SEM. n = 4. $^{\#}P<0.05$ *vs* the control group; $^{***}P<0.001$ *vs* the insulin-treated group.

4.4. Molecular docking models

Finally, we obtained the binding mode of the compound **P7** based on the docking simulation (PDB code 1Q6T). The model of compound **P7** showing in Figure 7 revealed that a dense network of hydrogen bonds are formed between the –N (CH_2COOCH_3) SO_2CH_3 group and the backbone NHs of Lys120, Ser216, Ala217 and Arg221 at the active site of PTP1B as well as hydrogen-bond interactions between oxygen atoms of alkoxy group and Arg47. In addition, hydrophobic and π-π

stacking interactions are formed between the phenyl ring of the ligand and the surrounding amino acid side chains. Due to these multiple interactions, compound **P7** effectively combined with the pockets to serve as a potent inhibitor.

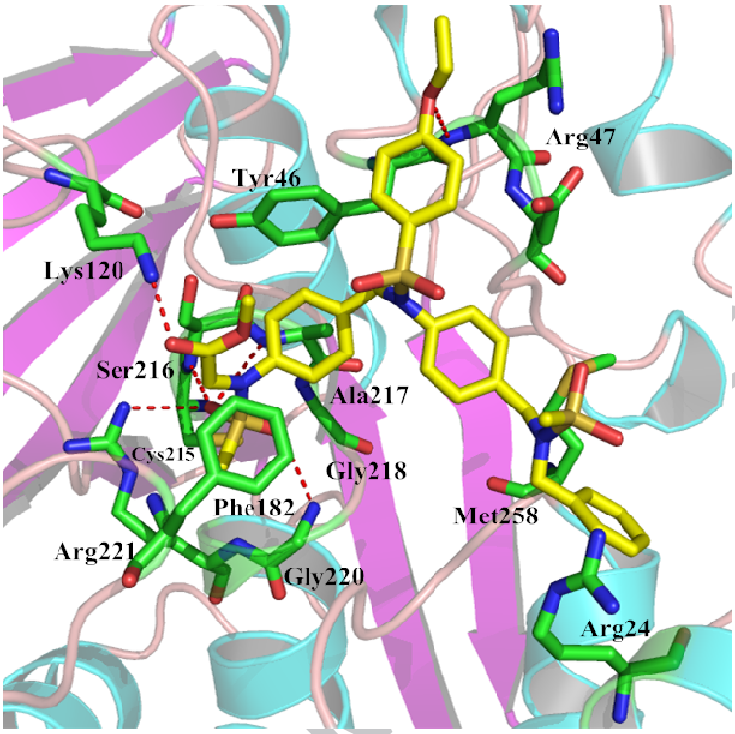


Figure 7. Binding model of compound **P7** to PTP1B. Carbon is in green for PTP1B and yellow for compound **P7**, oxygen atoms are in red, nitrogen is in blue, sulfur is in yellow. Hydrogen bonds are depicted with red dashes. This structure figure was prepared using PyMol.

5. Conclusion

In summary, the novel scaffold and simple synthesis method inhibitors were designed and synthesized and their biological properties were evaluated. The SAR studies of R_1-R_4 substituents of the chemical formula **4** revealed that six representative compounds (**P6**, **P7**, **P8**, **P9**, **P13**, **P15**) showed excellent inhibitory activity with IC_{50} values less than 1 μM. Among them, **P6**, **P7**, **P13**, **P15** were selected to investigate the selectivity demonstrated moderate selectivity over TCPTP. More importantly, compound **P7** exhibited high inhibitory activity (IC_{50} = 222 nM) with 8-fold selectivity over TCPTP through interacting with the A, B and C binding sites of PTP1B enzyme. Further studies on cellular activities revealed that compound **P7** could enhance insulin-mediated IRβ phosphorylation and insulin-stimulated glucose uptake. These novel compounds reported in this study could provide us a possible opportunity for further optimizing to design more potent and selective ABC type PTP1B inhibitors in future studies.

6. Experimental section

6.1. General synthetic methods

Reactions were monitored by thin-layer chromatography (TLC) on silica gel plates .^{1}H (400 MHz) NMR and ^{13}C (101 MHz) NMR spectra were recorded on a Bruker AVANCE II Fourier transform spectrometer. The chemical shifts were given in δ (ppm) refer to the signal of $CDCl_3$ (δ 7.26, ^{1}H NMR and δ 77.16, ^{13}C NMR) and the signal of $(CD_3)_2SO$ (δ 2.54, ^{1}H NMR and δ 39.52, ^{13}C NMR). Elemental analyses were performed on a Der C, H, N, O, S Elementar Analysen-systemeVario EL III.

Unless stated otherwise, Most of the chemicals and reagents used were of AR grade and were purchased from commercial suppliers without further purification. The solvents used were all AR grade and were redistilled in the presence of proper desiccant when necessary.

6.1.1. [(4-Bromomethyl-phenyl)-methanesulfonyl-amino]-acetic acid methyl ester (16a)

Methanesulfonyl chloride (3.41 mL, 44 mmol) was added dropwise to p-toluidine (4.28 g, 40 mmol) and pyridine (3.54 mL, 44 mmol) in DCM (50 mL) at 0°C under nitrogen. After addition, the mixture was warmed to room temperature and stirred overnight. The reaction was quenched with NaOH (6 mol/L) and enough water added to dissolve the resultant anion. The layers were separated and the aqueous layer washed with DCM (20 mL). The aqueous layer was cooled (0°C) and acidified to pH 2.0 by using 18% aqueous HCl. The product precipitates were filtered and washed with cold water and air-dried to constant weight to afford 4.68 g of **14a** (57%) as a colorless solid.

The reaction mixture of **14a** (4.26 g, 23 mmol) and suspension of NaH (722 mg, 30 mmol) in DMF (10 mL) was stirred at 0°C for 45 min. Then methyl bromoacetate (3 mL, 32.4 mmol) was added and allowed to warm to room temperature with stirring overnight. The solids were removed by filtration and washed with ethyl acetate (200 mL). The filtrate was washed with saturated brine. And the organic phase was concentrated under vacuum to afford the yellow oily liquid. Then purified using flash chromatography. Clean product fractions and mixed fractions eluted. The product fractions were combined and concentrated under vacuum to afford 5.22 g (88%) of **15a** as a white solid.

NBS (3.98 g, 22.4 mmol) and AIBN (50 mg) were added to a solution of **15a** (5.22 g, 20.3 mmol) in CCl_4 (50 mL) at room temperature. The mixture was thoroughly purged with N_2 before the

addition of AIBN to remove H_2O and O_2. The solution was stirred at 76°C under nitrogen for 10 h. The solution was filtered and the precipitate was washed with a little CCl_4. The combined filtrate was reduced in volume to give a light yellow liquid. Then purified using flash chromatography. Clean product fractions and mixed fractions eluted. The product fractions were combined and concentrated under vacuum to afford 4.77 g (70%) of **16a** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6,) δ 7.52 – 7.38 (m, 4H), 4.70 (s, 2H), 4.54 (m, 2H), 3.63 (s, 3H), 3.14 – 3.05 (m, 3H).

6.1.2. N-(4-Nitro-benzyl)-methanesulfonamide (8)

According to the same procedure described for **15a** to **16a**, 4-nitrotoluene (15 g, 110 mmol) was treated with NBS (19.58 g, 110 mmol) and benzoyl peroxide to afford 15.68 g (66%) of **5** as a light yellow solid. The reaction mixture of **5** (10 g, 46.3 mmol), potassium phthalimide (11.11 g, 60 mmol) and TBAB (50 mg, 0.16 mmol) in DMF (30 mL) was stirred at 76°C for 5 hours. The samples were withdrawn at different time intervals and analyzed using TLC until the reaction was completed. Then the reaction was quenched and cooled to room temperature. The solids were removed by filtration and washed with ethyl acetate (140 mL). The filtrate was washed with saturated brine, and the organic phase was concentrated under vacuum to afford 9.80 g (75%) of the yellow solid **6.**

The reaction mixture of **6** (9.82 g, 34.8 mmol) and hydrazine hydrate (6.7 mL) in methanol (50 mL) was stirred at 60°C for 3 h. The solvent was evaporated under vacuum and the residue dissolved in DCM (50 mL). The solution was extracted with (1 mol/L) NaOH (50 mL) and the organic layer was dried with Na_2SO_4 and concentrate in vacuum. The residue was purified by flash chromatography to afford 3.96 g (75%) of **7**. According to the same procedure described for **14**, methanesulfonyl chloride (2.25 mL, 29 mmol) was treated with **7** (3.96 g, 26 mmol) to afford 4.80 g (80%) of **8** as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 8.4 Hz, 2H), 7.78 (t, J = 6.0 Hz, 1H), 7.61 (d, J = 8.0 Hz, 2H), 4.30 (d, J = 6.4 Hz, 2H), 2.92 (s, 3H).

6.1.3. ({4-[(Benzenesulfonyl-{4-[(benzyl-methanesulfonyl-amino)-methyl]-phenyl}-amino)-methyl]-phenyl}-methanesulfonyl-amino)-acetic acid methyl ester (P5)

To a stirred solution of **8** (500 mg, 2.17 mmol) in DMF (12 mL) at ambient temperature was added benzyl bromide (0.31 mL, 2.61 mmol) and potassium carbonate (600 mg, 4.34 mmol) at room temperature. After 3 h, the reaction mixture was filtrated and washed with ethyl acetate (140 mL). The filtrate was washed with saturated brine and dried over anhydrous Na_2SO_4 and the organic phase

was concentrated under vacuum to afford the yellow solid. Then the resulting solid was recrystallized from ethanol and the pale yellow crystals **9a** were removed by filtration (523 mg, 75%).

$NaBH_4$ (197 mg, 5.2 mmol) was added to a mixture of **9a** (481 mg, 1.5 mmol), $NiCl_2 \cdot 6H_2O$ (618 mg, 2.6 mmol) and methanol at 0°C. The whole was stirred at room temperature for 30 min. After removal of methanol, the reaction mixture was acidified to pH 2.0 by using 10% aqueous HCl. It was then made basic with a 28% NH_3 aqueous solution and extracted with ethyl acetate. The organic layer was washed with saturated brine solution and dried over anhydrous Na_2SO_4. After removal of the solvent, further purification was done by column chromatography to give 399 mg (92%) of **10a**.

Pyridine (73 µL, 0.90 mmol) and DMAP were added to a solution **10a** (203 mg, 0.7 mmol) in anhydrous DCM (10 mL). Then benzenesulfonyl chloride (145 mg, 0.76 mmol) was dissolved in DCM and added dropwise under a nitrogen atmosphere. The reaction mixture was then stirred at room temperature for 1 days. Whereupon, HCl (1mol/ L) was added and the reaction mixture was extracted with DCM. The organic phases were combined and dried over anhydrous Na_2SO_4. After removal of volatiles, a residue was obtained that was then subjected to silica-gel column chromatographic purification to afford this intermediate **13a** (196 mg, 63%).

To a stirred solution of **13a** (178 mg, 0.40 mmol) in DMF (12 mL) at ambient temperature was added **16a** (155 mg, 0.46 mmol) and potassium carbonate (276 mg, 2.0 mmol) and potassium iodide. After one night, the reaction mixture was filtrated and washed with ethyl acetate (140 mL). The filtrate was washed with saturated brine and dried over anhydrous Na_2SO_4 and the organic phase was concentrated under vacuum to afford the yellow solid. Further purification was done by column chromatography (152 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 – 6.95 (m, 18H), 4.73 (s, 2H), 4.41 (s, 2H), 4.29 (d, J = 5.7 Hz, 4H), 3.76 (s, 3H), 3.07 (s, 3H), 2.76 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.6, 139.3, 137.8, 137.6, 136.3, 136.2, 135.7, 133.3, 129.3, 128.8, 128.5, 128.3, 128.2, 128.1, 127.4, 127.3, 127.2, 52.8, 52.0, 51.9, 51.1, 50.4, 38.9, 38.2. Anal. ($C_{32}H_{35}N_3O_8S_3$): Analysis Calcd for N, 6.13; C, 56.04; S, 14.03; H, 5.14. Found: N, 5.969; C, 55.95; S, 14.54; H, 4.736.

6.1.4. [(4-{[{4-[(Benzyl-methanesulfonyl-amino)-methyl]-phenyl}-(4-methoxy-benzenesulfonyl) -amino]-methyl}-phenyl)-methanesulfonyl-amino]-acetic acid methyl ester (P6)

The reaction mixture of phenol (3 g, 32 mmol) and KOH (2.69 g, 48 mmol) in methanol (40 mL) was stirred at 0°C under nitrogen for 15 min, then dimethylsulfate (6.06 g, 48 mmol) was added dropwise in the reaction and the mixture was gradually heated to 60°C. The samples were withdrawn at different time intervals and analyzed using TLC until the reaction was completed. Then it was cooled to room temperature, poured into water, and extracted into ethyl acetate. The organic layer was washed with water and 10% NaOH and dried over anhydrous Na_2SO_4. And the organic phase was concentrated under vacuum to afford the colorless liquid as product. Further purification was done by column chromatography affording 1.94 g (56%) of **11a** as a colorless oil.

11a（1.84 g, 17 mmol）was dissolved in DCM (50 mL). Then chlorosulfonic acid (2.97 g, 25.5 mmol) in DCM (20 mL) was added over 60 min at -5°C. The mixture was allowed to warm to room temperature with stirring additional 60 min. The mixture was poured into ice water and the organic phase was separated, washed with Na_2CO_3, $NaHCO_3$, water and saturated brine. It was finally dried over anhydrous Na_2SO_4, filtered and evaporated, affording in **12a** (2.11 g, 60%). According to the same procedure described for **13a**, **10a** (203 mg, 0.7 mmol) was treated with **12a** (157 mg, 0.76 mmol) to afford 228 mg (71%) of **13b** as a white solid. According to the same procedure described for **P6**, **13b** (184 mg, 0.40 mmol) was treated with **16a** (155 mg, 0.46 mmol) to afford 173 mg (61%) of **7** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49 (d, J = 8.8 Hz, 2H), 7.36 – 7.05 (m, 13H), 7.01 (d, J = 8.4 Hz, 2H), 4.73 (s, 2H), 4.45 (s, 2H), 4.23 (d, J = 14.8 Hz, 4H), 3.85 (s, 3H), 3.58 (s, 3H), 3.03 (s, 3H), 2.88 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.7, 162.8, 139.3, 138.1, 136.3, 136.0, 135.8, 129.6, 129.2, 128.8, 128.6, 128.3, 128.3, 128.1, 127.5, 127.2, 114.4, 55.8, 52.7, 52.0, 51.9, 51.1, 50.4, 38.9, 38.3. Anal. ($C_{33}H_{37}N_3O_9S_3$): Analysis Calcd for N, 5.87; C, 55.37; S, 13.44; H, 5.21. Found: N, 5.865; C, 55.08; S, 13.33; H, 4.829.

6.1.5. [(4-{[{4-[(Benzyl-methanesulfonyl-amino)-methyl]-phenyl}-(4-ethoxy-benzenesulfonyl)-amino]-methyl}-phenyl)-methanesulfonyl-amino]-acetic acid methyl ester (P7)

The phenol (1.88 g, 20 mmol) was dissolved in a mixture of DMF (50 mL) and potassium carbonate (4.14 g，30 mmol), Then the bromoethane (2.3 mL, 30 mmol) was added and the mixture was stirred for about 5 hours with heating and reflux at 70°C. The solids were removed by filtration and washed with ethyl acetate (140 mL). The filtrate was washed with a saturated brine solution and dried over anhydrous Na_2SO_4, and the organic phase was concentrated under vacuum to afford 1.72 g (70 %) of **11b** as a colorless oil. According to the same procedure described for **12a**, **11b** (1.47 g,

12 mmol) was treated with chlorosulfonic acid (2.10 g, 18 mmol) to afford 1.41 g (53%) of **12b** as a colorless oil. According to the same procedure described for **13a**, **10a** (203 mg, 0.7 mmol) was treated with **12b** (168 mg, 0.76 mmol) to afford 236 mg (71%) of **13c** as a red oil. According to the same procedure described for **P6**, **13c** (190 mg, 0.40 mmol) was treated with **16a** (155 mg, 0.46 mmol) to afford 170 mg (58%) of **P7** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.8 Hz, 2H), 7.40 – 7.29 (m, 7H), 7.26 – 7.13 (m, 4H), 7.01 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 4.71 (s, 2H), 4.40 (s, 2H), 4.29 (d, *J* = 6.0 Hz, 4H), 4.12 (q, *J* = 6.8Hz, 2H), 3.76 (s, 3H), 3.07 (s, 3H), 2.75 (s, 3H), 1.48 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.6, 162.1, 139.2, 138.1, 136.3, 136.0, 135.8, 129.6, 128.9, 128.8, 128.5, 128.3, 128.2, 128.1, 127.4, 127.2, 114.8, 63.8, 52.7, 52.0, 51.9, 51.0, 50.3, 38.9, 38.3, 14.5. Anal. ($C_{34}H_{39}N_3O_9S_3$): Analysis Calcd for N, 5.76; C, 55.95; S, 13.18; H, 5.39. Found: N, 5.662; C, 55.93; S, 13.70; H, 4.81.

6.1.6. [(4-{[{4-[(Benzyl-methanesulfonyl-amino)-methyl]-phenyl}-(4-propoxy-benzenesulfonyl)-amino]-methyl}-phenyl)-methanesulfonyl-amino]-acetic acid methyl ester (P8)

According to the same procedure described for **11b**, phenol (1.88 g, 20 mmol) was treated with 1-bromopropane (2.7 mL, 30 mmol) to afford 1.95 g (72%) of **11c** as a colorless oil. According to the same procedure described for **12b**, **11c** (1.63 g, 12 mmol) was treated with chlorosulfonic acid (2.10 g, 18 mmol) to afford 1.42 g (50%) of **12c** as a colorless oil. According to the same procedure described for **13b**, **10a** (203 mg, 0.7 mmol) was treated with **12b** (178 mg ,0.76 mmol) to afford 195 mg (60%) of **13d**. According to the same procedure described for **6**, **13d** (195 mg, 0.40 mmol) was treated with **16a** (155 mg, 0.46 mmol) to afford 157 mg (53%) of **P8** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (d, *J* = 8.8 Hz, 2H), 7.35 – 6.99 (m, 15H), 4.74 (s, 2H), 4.46 (s, 2H), 4.24 (d, *J* = 14.4 Hz, 4H), 4.03 (t, *J* = 6.4 Hz, 2H), 3.59 (s, 3H), 3.04 (s, 3H), 2.89 (s, 3H), 1.82 – 1.70 (m, 2H), 1.00 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.6, 162.2, 139.2, 138.1, 136.3, 136.0, 135.8, 129.6, 128.9, 128.7, 128.5, 128.3, 128.2, 128.1, 127.4, 127.2, 114.8, 69.6, 52.6, 52.0, 51.9, 51.0, 50.3, 38.9, 38.3, 21.9, 10.3. Anal. ($C_{35}H_{41}N_3O_9S_3$): Analysis Calcd for N, 5.65; C, 56.51; S, 12.93; H, 5.56. Found: N, 5.807; C, 56.60; S, 13.82; H, 5.466.

6.1.7. [(4-{[{4-[(Benzyl-methanesulfonyl-amino)-methyl]-phenyl}-(4-butoxy-benzenesulfonyl)-amino]-methyl}-phenyl)-methanesulfonyl-amino]-acetic acid methyl ester (P9)

According to the same procedure described for **11b**, phenol (1.88 g, 20 mmol) was treated with 1-bromobutane (3.2 mL, 30 mmol) afford 2.17 g (72%) of **11d** as a colorless oil. According to the

same procedure described for **12a**, **11d** (1.80 g, 12 mmol) was treated with chlorosulfonic acid (2.10 g, 8 mmol) to afford 1.53 g (54%) of **12d** as a colorless oil. According to the same procedure described for **13a**, **10a** (203 mg, 0.7 mmol) was treated with **12d** (178 mg, 0.76 mmol) to afford 204 mg (58%) of **13e**. According to the same procedure described for **P6**, **13e** (201 mg, 0.40 mmol) was treated with **16a** (155 mg, 0.46 mmol) to afford 167 mg (55%) of **P9** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (d, J = 7.6 Hz, 2H), 7.40 – 6.95 (m, 15H), 4.74 (s, 2H), 4.46 (s, 2H), 4.24 (d, J = 14.0Hz, 4H), 4.07 (s, 2H), 3.59 (s, 3H), 3.04 (s, 3H), 2.89 (s, 3H), 1.73 (s, 2H), 1.45 (d, J = 6.8Hz, 2H), 0.95 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.6, 162.2, 139.2, 138.1, 136.3, 136.0, 135.8, 129.5, 128.9, 128.7, 128.5, 128.3, 128.2, 128.1, 127.4, 127.2, 114.8, 67.8, 52.6, 52.0, 51.9, 51.0, 50.3, 38.9, 38.3, 30.5, 18.7, 13.7. Anal. ($C_{36}H_{43}N_3O_9S_3$): Analysis Calcd for N, 5.54; C, 57.05; S, 12.69; H, 5.72. Found: N, 5.548; C, 56.84; S, 13.03; H, 5.609.

6.1.8. ({4-[({4-[(Benzyl-methanesulfonyl-amino)-methyl]-phenyl}-phenylmethanesulfonyl-amino)-methyl]-phenyl}-methanesulfonyl-amino)-acetic acid methyl ester (P10)

According to the same procedure described for **13a**, **10a** (203 mg, 0.7 mmol) was treated with benzylsulfonyl chloride (145 mg, 0.76 mmol) to afford 197 mg (63%) of **13f**. According to the same procedure described for **P6**, **13f** (178 mg, 0.40 mmol) was treated with **16a** (155 mg, 0.46 mmol) to afford 141 mg (50%) of **P10** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.44 – 7.31 (m, 7H), 7.25 – 7.11 (m, 11H), 4.80 (s, 2H), 4.56 (s, 2H), 4.46 (s, 2H), 4.26 (d, J = 9.6 Hz, 4H), 3.60 (s, 3H), 3.04 (s, 3H), 2.91 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.6, 139.3, 138.3, 136.3, 136.1, 135.7, 131.0, 129.1, 128.8, 128.5, 128.4, 128.4, 128.3, 128.0, 127.4, 127.2, 55.9, 53.3, 52.0, 51.9, 51.0, 50.3, 38.8, 38.4. Anal. ($C_{33}H_{37}N_3O_8S_3$): Analysis Calcd for N, 6.00; C, 56.63; S, 13.74; H, 5.33. Found: N, 5.879; C, 56.59; S, 14.52; H, 4.919.

6.1.9. [Methanesulfonyl-(4-{[(4-{[methanesulfonyl-(4-methyl-benzyl)-amino]-methyl}-phenyl)-phenylmethanesulfonyl-amino]-methyl}-phenyl)-amino]-acetic acid methyl ester (P11)

According to the same procedure described for **9a**, **8** (500 mg, 2.17mmol) was treated with 1-bromomethyl-4-methyl-benzene (352 μL, 2.61 mmol) to afford 531 mg (73%) of **9b** as a yellow solid. According to the same procedure described for **10a**, **9b** (481 mg, 1.5 mmol) was treated with $NiCl_2{\cdot}6H_2O$ (618 mg, 2.6 mmol), $NaBH_4$ (198 mg, 5.2 mmol) to afford 409 mg (90%) of **10b**. According to the same procedure described for **13a**, **10b** (213 mg, 0.7 mmol) was treated with benzylsulfonyl chloride (145 mg, 0.76 mmol) to afford 187 mg (58%) of **13g**. According to the same

procedure described for **P6**, **13b** (183 mg, 0.40 mmol) was treated with **16a** (155 mg, 0.46 mmol) to afford 146 mg (51%) of **P11** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 – 7.29 (m, 7H), 7.28 – 6.98 (m, 10H), 4.80 (s, 2H), 4.56 (s, 2H), 4.46 (s, 2H), 4.21 (s, 4H), 3.60 (s, 3H), 3.04 (s, 3H), 2.88 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.6, 139.3, 138.4, 136.7, 136.1, 135.7, 133.2, 131.0, 129.1, 128.9, 128.8, 128.5, 128.4, 128.4, 128.0, 127.2, 55.9, 53.3, 52.0, 51.9, 50.4, 49.9, 38.8, 38.6, 20.7. Anal. ($C_{34}H_{39}N_3O_8S_3$): Analysis Calcd for N, 5.89; C, 57.20; S, 13.47; H, 5.51. Found: N, 6.225; C, 57.13; S, 13.31; H, 5.621.

6.1.10. [(4-{[(4-{[(3-Chloro-benzyl)-methanesulfonyl-amino]-methyl}-phenyl)-phenylmethanesulfonyl-amino]-methyl}-phenyl)-methanesulfonyl-amino]-acetic acid methyl ester (P12)

According to the same procedure described for **14a**, 4-methyl-aniline (4.29 g, 40 mmol) was treated with ethanesulfonyl chloride (5.66 g, 44 mmol) to afford 6.4 g (80%) of **14b** as a colorless solid. According to the same procedure described for **15a**, **14b** (4.58 g, 23 mmol) was treated with methyl bromoacetate (3 mL, 32mmol) to afford 4.926 g (77%) of **15b** as a white solid. According to the same procedure described for **16a**, **15b** (4.07 g, 15 mmol) was treated with NBS (2.94 g, 16.5 mmol) and AIBN to afford 3.55 g (70%) of **16b** as a white solid. According to the same procedure described for **9a**, **8** (500 mg, 2.17 mmol) was treated with 3-chlorobenzyl bromide (536 mg, 2.61 mmol) to afford 556 mg (72 %) of **9c**. According to the same procedure described for **10a**, **9c** (532 mg, 1.5 mmol) was treated with $NiCl_2{\cdot}6H_2O$ (618 mg, 2.6 mmol), $NaBH_4$ (197 mg, 5.2 mmol) to afford 430 mg (88%) of **10c**. According to the same procedure described for **13a**, **10c** (227 mg, 0.7 mmol) was treated with benzylsulfonyl chloride (145 mg, 0.76 mmol) to afford 189 mg (56%) of **13h**. According to the same procedure described for **P6**, **13h** (192 mg, 0.40 mmol) was treated with **16b**(155 mg, 0.46 mmol) to afford 156 mg (52%) of **P12** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 – 6.97 (m, 17H), 4.78 (s, 2H), 4.54 (s, 2H), 4.47 (s, 2H), 4.29 (d, J = 10.4 Hz, 4H), 3.58 (s, 3H), 3.17 (q, J = 7.3 Hz, 2H), 2.95 (s, 3H), 1.17 (t, J = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4, 139.8, 138.9, 137.6, 136.6, 135.1, 134.7, 131.0, 130.2, 129.5, 129.3, 129.2, 129.1, 128.8, 128.7, 128.6, 128.4, 128.2, 126.9, 57.9, 54.9, 53.0, 52.6, 50.4, 50.3, 47.4, 40.1, 8.0. Anal. ($C_{34}H_{38}ClN_3O_8S_3$): Analysis Calcd for N, 5.62; C, 54.57; S, 12.85; H, 5.12. Found: N, 5.64; C, 54.43; S, 12.98; H, 4.604.

6.1.11. {[4-({[4-(Benzyl-methanesulfonyl-amino)-phenyl]-phenylmethanesulfonyl-amino}-methyl)-phenyl]-methanesulfonyl-amino}-acetic acid methyl ester (P13)

According to the same procedure described for **8**, p-nitroaniline (3.59 g, 26 mmol) was treated with methanesulfonyl chloride (2.24 mL, 29 mmol) to afford 4.68 g (83%) of **17** a yellow solid. According to the same procedure described for **9a**, **17** (469 mg, 2.17 mmol) was treated with benzyl bromide (310 μL, 2.61 mmol) to afford 500 mg (75.3%) of **18a** as a white solid. According to the same procedure described for **10a**, **18a** (460 mg, 1.5 mmol) was treated with $NiCl_2 \cdot 6H_2O$ (618 mg, 2.6 mmol), $NaBH_4$ (197 mg, 5.2 mmol) to afford 375 mg (90.5%) of **19b**. According to the same procedure described for **13a**, **19b** (194 mg, 0.7 mmol) was treated with benzylsulfonyl chloride (145 mg, 0.76 mmol) to afford 182 mg (60%) of **20a** as a white solid. According to the same procedure described for **P6**, **20a** (172 mg, 0.40 mmol) was treated with **16a** (155 mg, 0.46 mmol) to afford 154 mg (56%) of **P13** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.06 (m, 18H), 4.82 (s, 2H), 4.47 (s, 2H), 4.40 (s, 2H), 4.31 (s, 2H), 3.82 – 3.71 (m, 3H), 3.07 (s, 3H), 2.94 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 169.7, 139.3, 138.2, 138.0, 136.6, 136.0, 131.0, 129.0, 128.6, 128.5, 128.3, 128.3, 127.8, 127.4, 127.1, 56.2, 53.5, 53.2, 52.1, 51.9, 38.8, 37.4. Anal. ($C_{32}H_{35}N_3O_8S_3$): Analysis Calcd for N, 6.13; C, 56.04; S, 14.03; H, 5.14. Found: N, 6.29; C, 56.02; S, 14.03; H, 4.869.

6.1.12. ({4-[({4-[(3-Chloro-benzyl)-methanesulfonyl-amino]-phenyl}-phenylmethanesulfonyl-amino)-methyl]-phenyl}-methanesulfonyl-amino)-acetic acid methyl ester (P14)

According to the same procedure described for **18a**, **17** (469 mg, 2.17 mmol) was treated with 3-chlorobenzyl bromide (536 mg, 2.61 mmol) to afford 546 mg (74%) of **18b** as a yellow solid. According to the same procedure described for **10a**, **18b** (511 mg, 1.5 mmol) was treated with $NiCl_2 \cdot 6H_2O$ (618 mg, 2.6 mmol), $NaBH_4$ (197 mg, 5.2 mmol) to afford 408 mg (88%) of **19b**. According to the same procedure described for **13a**, **19b** (218 mg, 0.7 mmol) was treated with benzylsulfonyl chloride (145 mg, 0.76 mmol) to afford 184 mg (57%) of **20b** as a white solid. According to the same procedure described for **P6**, **20b** (186 mg, 0.40 mmol) was treated with **16a** (155 mg, 0.46 mmol) to afford 147 mg (51%) of **P14** as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.45 – 7.11 (m, 17H), 4.81 (d, *J* = 10.8 Hz, 4H), 4.61 (s, 2H), 4.46 (s, 2H), 3.62 (s, 3H), 3.13 – 2.99 (m, 6H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 169.7, 139.4, 139.3, 138.4, 137.8, 136.1, 133.0, 131.0, 130.3, 129.0, 128.5, 128.5, 128.4, 128.3, 128.3, 127.5, 127.4, 127.1, 126.5, 56.2, 53.2, 52.9, 52.1, 51.9, 38.8, 37.4. Anal. ($C_{32}H_{34}ClN_3O_8S_3$): Analysis Calcd for N, 5.83; C, 53.36; S, 13.36; H, 4.76. Found: N, 5.724; C, 53.08; S, 13.62; H, 4.409.

6.1.13. {Ethanesulfonyl-[4-({[4-(4-ethoxy-benzenesulfonylamino)-phenyl]-phenylmethanesulfonyl-amino}-methyl)-phenyl]-amino}-acetic acid methyl ester (P15)

According to the same procedure described for **13a**, p-nitroaniline (2.76 g, 20 mmol) was treated with benzylsulfonyl chloride (4.20 g, 22 mmol) to afford 2.65g (45%) of **21a**. According to the same procedure described for **P6**, **21a** (292 mg, 1 mmol) was treated with **16b** (385 mg, 1.1 mmol) to afford 309 mg (55%) of **22a**. According to the same procedure described for **10a**, **22a** (281 mg, 0.5 mmol) was treated with $NiCl_2{\cdot}6H_2O$ (214 mg, 0.9 mmol), $NaBH_4$ (68 mg, 1.8 mmol) to afford 253 mg (95%) of **23a**. According to the same procedure described for **13a**, **23a** (213 mg, 0.4 mmol) was treated with **12b** (97 mg, 0.44 mmol) to afford 158 mg (56%) of **P15** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.42 – 7.28 (m, 7H), 7.16 (d, J = 8.0 Hz, 2H), 7.10 (d, J = 8.4 Hz, 2H), 7.03 (d, J = 8.4Hz, 2H), 6.95 (d, J = 8.4 Hz, 2H), 4.70 (s, 2H), 4.52 (s, 2H), 4.46 (s, 2H), 4.06 (m, 2H), 3.59 (s, 3H), 3.16 (m, 2H), 1.30 (t, J = 6.8 Hz, 3H), 1.15 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4, 162.9, 139.7, 136.5, 136.4, 135.4, 130.9, 130.3, 129.7, 129.5, 129.5, 129.1, 129.0, 128.6, 128.1, 121.2, 114.8, 64.2, 57.7, 55.1, 53.0, 52.6, 47.4, 14.7, 8.0. Anal. ($C_{33}H_{37}N_3O_9S_3$): Analysis Calcd for N, 5.87; C, 55.37; S, 13.44; H, 5.21. Found: N, 5.681; C, 55.12; S, 13.91; H, 4.774.

6.1.14. {[4-({[4-(Benzenesulfonylmethyl-amino)-phenyl]-phenylmethanesulfonyl-amino}-methyl)-phenyl]-ethanesulfonyl-amino}-acetic acid methyl ester (P16)

According to the same procedure described for **P15**, **23b** (236 mg, 0.4 mmol) was treated with benzylsulfonyl chloride (84 mg, 0.44 mmol) to afford 149 mg (50%) of **P16** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.90 (s, 1H), 7.66 – 6.89 (m, 17H), 4.73 (s, 2H), 4.41 (d, J = 3.2Hz, 4H), 4.07 (t, J = 6.4 Hz, 2H), 3.59 (s, 3H), 3.03 (s, 3H), 1.84 – 1.63 (m, 2H), 1.45 (m, 2H), 1.06 – 0.83 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.6, 162.2, 139.3, 137.8, 135.9, 133.8, 130.9, 129.6, 129.3, 129.2, 129.0, 128.6, 128.3, 128.2, 127.2, 118.6, 114.8, 67.8, 57.3, 52.8, 52.0, 51.9, 38.8, 30.6, 18.7, 13.7. Anal. ($C_{35}H_{41}N_3O_9S_3$): Analysis Calcd for N, 5.65; C, 56.51; S, 12.93; H, 5.56. Found: N, 5.71; C, 55.88; S, 13.68; H, 4.683.

6.2. PTP1B and related PTPs biological assay

PTP1B inhibitory activities were measured as previously described with minor revision.[34, 39] Briefly, PTP1B hydrolyzes pNPP to pNP that can be detected at 405 nm. Test compounds were predispensed in 96-well microplates as 1.0 μL aliquots per well in 100% DMSO. The PTP1B enzymatic assay was carried out in a total volume of 100 μL per well in assay plates with 15 nM recombinant PTP1B protein, 2 mM p-nitrophenylphosphonic acid (pNPP), 1 mM dithiothreitol and 1

mM EDTA (pH 6.5). After 30 min incubation at 37°C, the reaction was terminated by addition of 2.5 M NaOH. The amount of hydrolysis product, pNP, was monitored by detection of the absorbance at 405 nm. To determine cross-reactivity of test compounds, the same protocol was followed for inhibition assay with TCPTP. To study the inhibition on the other PTPs family members, SHP1, SHP2 and LAR assays were performed according to procedures described previously.

6.3. Effect of PTP1B inhibitors on the phosphorylation level of IR in transfected CHO cells and 3T3-L1 cells

CHO cells were cultured in the presence of F12 supplemented with 10% FBS and seeded 24 h before transfection in 6 cm dish (6×10^5 cells per dish). 2μg of the human IR plasmid was introduced into cells by using Fu GENE 6 transfection reagent (Roche) according to the manufacturer's protocol. Cells were transfected for 8 h, and harvested with 0.05% trypsin and 0.02% EDTA prior to reseeding onto a 6-well plate. After 24 h with 5% CO_2 at 37°C, CHO cells serum free starved for 2 h were incubated with compounds for 4 h, then 10 nM insulin was added stimulation for 10 min before harvested.

For total protein extraction, CHO cells were lysed in the ice-cold buffer A (1% Triton X-100, 100 mM sodium pyrophosphate, 2 mM phenylmethylsulfonyl fluoride (PMSF), 50 mM HEPES, pH 7.4) and centrifuged at 15, 000 ×g for 20 min at 4℃. Supernatants were collected and stored at -80℃. For subcellular fractionation, cells were lysed in buffer B (0.25 M sucrose, 2 mM EDTA, 1 mM phenylmethylsulfony fluoride (PMSF) and 10 mM Tris-HCl, pH 7.4). The lysates were centrifuged at 750 ×g for 15 min, and the supernatants were centrifuged at 12, 000 ×g for 20 min to isolate the crude plasma membrane fraction as the pellets. Equal amount of protein from each sample were denatured and subjected to SDS-PAGE and blotted on PVDF membrane, which was incubated for 2 h at room temperature with blocking buffer (5% non-fat milk, 0.1% Tween 20, in TBS, pH 7.6) and then probed with primary antibodies (PY-20 and β-actin) overnight at 4℃. After incubation with the appropriate secondary antibodies, the immunoreactive band was detected by an ECL Western blotting detection system (GE Healthcare).

To determine the phosphorylation level of IRβ in 3T3-L1 cells, the total protein extraction of P7-treated cells were resolved by SDS-PAGE and immunoblotted with anti-pIRβ or anti-IRβ antibody.

6.4. Glucose uptake assay

2-NBDG is a fluorescent glucose analogue. Glucose uptake into L6 myotubes or human skeletal muscle cells (HSkMC) was measured using 2-NBDG as previously described.[40, 41] Briefly, L6 myoblasts were grown in 96-well fluorescent plates and differentiated as described above. HSkMC were also grown in 96-well fluorescent plates. Differentiated L6 myotubes or HSkMC were washed once with warm PBS, incubated in serum-free DMEM for 3 h at 37°C and then stimulated with either 10 nM insulin for 10 min at 37°C in Kreb's ringer phosphate buffer (128 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 1.25 mM $MgSO_4$, 10 mM $NaPO_4$, pH 7.4). Glucose uptake was initiated by the addition of 80 μM 2-NBDG to each well. After 30 min, the supernatant was removed. Plates were then rinsed with PBS and the fluorescence of the samples was monitored at an excitation wavelength of 485 nm and an emission wave length of 535 nm. To exclude false-positive, cells treated with drugs in the absence of 2-NBDG were measured and taken as the background. The relative fluorescence intensities minus the background were used for subsequent data analysis.

6.5. Molecular Docking

All the molecular docking into the pocket of PTP1B in the open conformation have been performed using the Surflex-Dock docking module within the software Sybyl X 1.0 with the default settings. The X-ray crystal structure of PTP1B (PDB code 1Q6T) was retrieved from the RCSB Protein Data Bank (http://www.rcsb.org).

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***Graphical Abstract:**