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Facile fabrication of promising protein tyrosine phosphatase (PTP) inhibitor entities based on 'clicked' serine/threonine–monosaccharide hybrids

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ABSTRACT

Protein tyrosine phosphatases (PTPs) are well-validated therapeutic targets for many human major diseases. The development of their potent inhibitors has therefore become a main focus of both academia and the pharmaceutical industry. We report herein a facile strategy toward the fabrication of new and competent PTP inhibitor entities by simply 'clicking' alkynyl amino acids onto diverse azido sugar templates. Triazolyl glucosyl, galactosyl, and mannosyl serine and threonine derivatives were efficiently synthesized via click reaction, which were then identified as potent CDC25B and PTP1B inhibitors selective over a panel of homologous PTPs tested. Their inhibitory activity and selectivity were found to largely lie on the structurally and configurationally diversified monosaccharide moieties whereon serinyl and threoninyl residues were introduced. In addition, MTT assay revealed the triazole-connected sugar-amino acid hybrids may also inhibit the growth of several human cancer cell lines including A549, Hela, and especially HCT-116. On the basis of such compelling evidence, we consider that this compound series could furnish promising chemical entities serving as new CDC25B and PTP1B inhibitors with potential cellular activity. Furthermore, the 'click' strategy starting from easily accessible and biocompatible amino acids and sugar templates would allow the modular fabrication of a rich library of new PTP inhibitors efficaciously and productively.

1. Introduction

The protein tyrosine phosphatases (PTPs) constitute a large class of functional enzymes governing the pivotal tyrosine phosphorylation processes on cellular level.[1] A myriad of biochemical studies indicated that the suppression of certain overexpressed PTPs with dysfunctions in vivo may lead to the treatment of many human major diseases.

For instance, the PTP1B-knockout mice model displayed increased insulin sensitivity and enhanced glycemic control, and was resistant to diet-induced obesity.[2,3] Meanwhile, several recent investigations suggest that PTP1B may qualify as a new therapeutic target for breast cancer.[4] The cell division cycle 25 (CDC25) phosphatases A, B, and C regulate cyclin-dependent kinases, the crucial component of the eukaryotic cell division cycle.[5a] Among the three isoforms, CDC25A and B have been identified to be overexpressed in a wide range of human cancers including breast, colon, cervix, lung, etc.[5b] This suggests that the inhibition of CDC25A and/or B may become a promising strategy in oncology.

Consequently, numerous programs have been initiated en route to the fabrication of potent and bioavailable small-molecule PTP inhibitors via both academia and the pharmaceutical industry.[6,7] The majority of these bioactive compounds are phosphotyrosine (pTyr) mimetics that competitively inhibit the targeted PTP. For example, the difluoromethylene phosphonate (DFMP), carboxylic acid and heterocyclic pTyr surrogates generally constitute the competitive inhibitor category of PTP1B.[8] Furthermore, noncompetitive inhibitors that induce the open conformation of WPD loop have furnished alternative insight for gaining its inhibition.[9] On the other hand, CDC25 phosphatase inhibitors discovered till date fall principally into quinoids whereas relatively fewer other bioactive compound sorts were identified.[5]

Nevertheless, most of the currently characterized PTPs inhibitors encounter limitations such as the unsatisfactory cellular activity and low bioavailability. In addition, several special compound series such as the quinonyl derivatives could release reactive oxygen species (ROS) which may uncertainly bring on toxicity to normal tissues. As a consequence, the discovery of new chemical

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entities competent to serve as PTP inhibitors remains quite desirable.

Sugars are pivotal regulators of numerous biological and pathological events in nature.[10a] These naturally abundant materials have also demonstrated their crucial roles in drug development.[10b,c] Becker and co-workers emphasized in a recent review that the introduction of pharmacophores onto sugar templates that possess dense stereochemical information is an excellent strategy for the development of bioactive compounds with rich structural diversity.[10b] Hence, the validation of a potent synthetic tool for efficiently constructing such glycoconjugates has become crucial.

Thanks to the definition of click chemistry[11a] and sequentially the identification of its representative reaction, the Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition[11b] by Sharpless and co-workers, numerous triazole-functionalized sugar derivatives with potential medicinal values were able to be modularly synthesized in the past decade.[12] 'Click-stitched' natural products such as sugar-amino acid hybrids were also independently prepared by Dondoni's[13a] and Rutjes's[13b] groups, which have particularly absorbed our interest.

Owning to its structural similarity to the reported isoxazole acid that mimics the PTP peptide substrate by *Abbott*'s laboratory,[14] the triazolyl amino acid residue is envisioned capable to serve as a new pTyr surrogate (Fig. 1). Moreover, since the pTyr itself is inadequate to achieve PTP inhibition, it is noted that the linkage of hydrophobic functionalities such as benzyl groups to pTyr could enhance the binding affinity as well as the cellular activity of the produced compounds.[6,8,9b] We have hence chosen several benzyl 1- or 6-azido monosaccharide epimers on which propargyl amino acids could be 'clicked'. As shown in Figure 1, the subsequently formed triazolyl amino acids presented on structurally and configurationally diverse benzyl sugar templates are deemed to possess distinct inhibitory potency and specificity on PTPs. Consequently, we report herein the preparation and biological studies of a new class of PTP inhibitors based on triazole-linked sugar-amino acid hybrids.

2. Results and discussion

2.1. Synthesis

As shown in Figure 1, to achieve the click reaction for the formation of the desired triazoles, the known benzyl 6-azido-6-deoxy-1-*O*-methoxy-α-D-glucoside (**1**), galactoside (**2**), mannoside (**3**), and 1-azido-1-deoxy-β-D-glucoside (**4**) were used as glycodonors.[15]

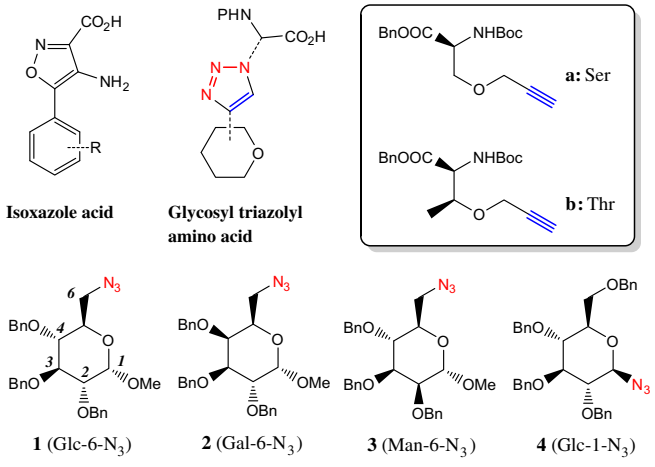


Figure 1. Heterocyclic acid-based PTPs inhibitors and alkynyl amino acid (**a** and **b**) and azido benzyl sugar templates (**1**–**4**) used for click reaction in this study.

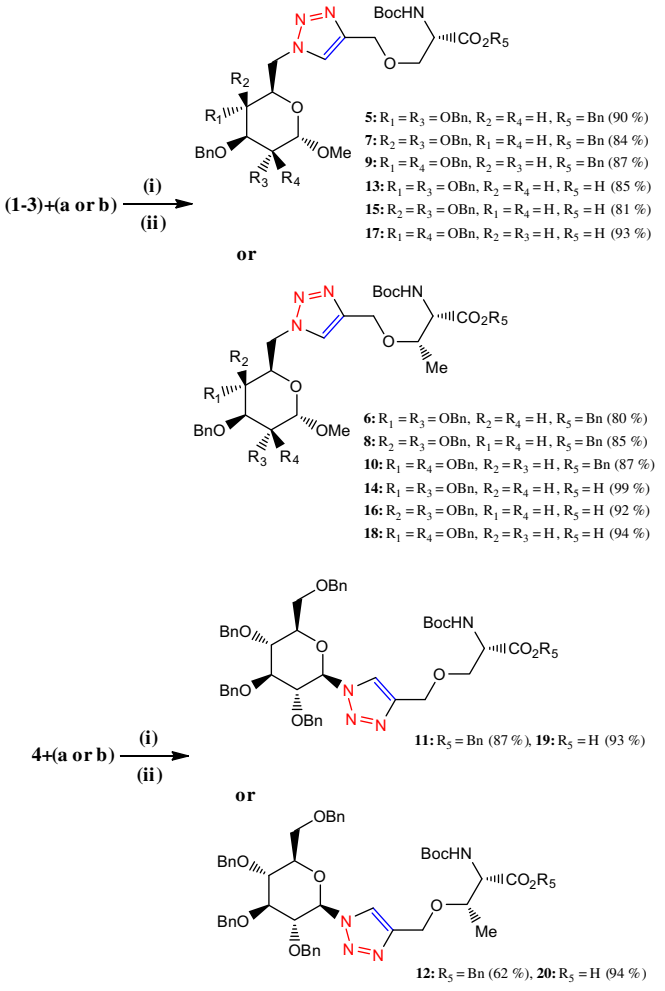
The *O*-propynyl serine and threonine **a** and **b** were prepared via a previously reported method in one-pot from commercially available Boc-L-serine and Boc-L-threonine.[16]

The Cu(I)-catalyzed *Huisgen* [3+2] cycloaddition between compounds **1**–**4** and **a** or **b** was sequentially performed in a solvent mixture of CH_2Cl_2/H_2O (1:1, v/v), shown in Scheme 1. To our delight, the click reactions of all azido glycosides with the alkynyl amino acids proceeded smoothly under the promotion of Na ascorbate/$CuSO_4{\cdot}5H_2O$ with vigorous stirring over 8 h at rt, affording the unique 1,4-disubstituted triazole-linked sugar–serine or threonine hybrids. The glucosyl, galactosyl and mannosyl 6-substituted serinyl and threoninyl triazoles **5**–**10** as well as the glucosyl 1-substituted serinyl triazole **11** were obtained in favorable yields of 80–90%. However, the glucosyl 1-substituted threoninyl triazole **12** was given by the click reaction of compound **4** with **b** less efficiently under the same condition in a moderate yield of 62%.

In order to furnish the desired carboxylic acid-exposed products with retained benzyl groups on sugar moieties, a Pd/C/H_2 system was used for the hydrogenolysis within a short timeframe of about 20 min.[16a] Benzyl esters **5**–**12** were therefore treated with such condition, affording selectively the desired free acids **13**–**20** in good-to-excellent yields (81–99%, Scheme 1).

2.2. Biological assay

The inhibitory activity of the prepared triazolyl serine– and threonine–sugar hybrids **13**–**20** on a panel of PTPs including



Scheme 1. Reagents and conditions: (i) Na ascorbate, $CuSO_4{\cdot}5H_2O$, CH_2Cl_2/H_2O (1:1, v/v), rt; (ii) Pd/C, H_2, MeOH, rt.

CDC25A, CDC25B, PTP1B, TCPTP, SHP-1, SHP-2, and LAR was sequentially assessed via our previously developed methods at a compound concentration of 100 μg/mL.[17]

Notably, despite the click chemistry has been well employed to establish triazolyl PTPs inhibitor libraries,[7a,18] the use of sugar templates whereon amino acid precursors were 'clicked' toward the same purpose remains much less explored. To our delight, the designed and prepared triazolyl serine– and threonine–sugar hybrids **13**–**20** showed micromole-ranged inhibitory activities as well as specificities on different PTPs, given in Table 1.

Interestingly, all compounds tested displayed preferable inhibitory effects on PTP1B and CDC25B, lesser inhibitory potency on TCPTP and CDC25A and almost no inhibition toward SHP-1, SHP-2, and LAR. Glucosyl 6-triazoloserine **13** ($IC_{50} \approx 70$ μM) and threonine **14** ($IC_{50} \approx 25$ μM) possess, respectively, almost identical IC_{50} values on PTP1B and CDC25B whereas the latter is about threefold more active than the former. This demonstrates that the additional methyl group on threonine residue contributes evidently to the inhibitory activity. Moreover, both compounds showed weak or no inhibitory activities on TCPTP, CDC25A, SHP, and LAR.

The galactosyl 6-triazoloserine **15** ($IC_{50} \approx 20$ μM) is similarly an equally potent inhibitor of both PTP1B and CDC25B, which displayed around 2.5-fold decreased potency toward TCPTP (IC_{50} = 55 μM) and no activity on CDC25A, SHP, and LAR. In contrast, its triazolothreoninyl counterpart **16** appeared to be a more specific PTP1B (IC_{50} = 15 μM) inhibitor with twofold decreased activity on CDC25B (IC_{50} = 31 μM) and no inhibitory effects on all of the rest PTPs tested (IC_{50} >120 μM). The mannosyl 6-triazoloserine **17** (IC_{50} = 8 μM) and threonine **18** (IC_{50} = 12 μM) are better CDC25B inhibitors having approximately 2-, 4-, and 10-fold selectivity over PTP1B, CDC25A and TCPTP, respectively, and do not inhibit the catalytic activities of SHP and LAR.

Notably, galactosyl triazoloserine **15** is almost 3.5-fold more potent than its glucosyl epimer **13**, which implies that the axial benzyl group on C4-position of galactoside is privileged in configuration over the C4-equatorial benzyl group of glucoside toward PTP1B and CDC25B inhibition. However, no obvious IC_{50} deficit is observed between threoninyl sugar C4-epimers **14** and **16**. Similarly, whereas only unapparent inhibitory difference was emerged between the threoninyl C2-epimers **14** and **18**, the serinyl mannoside **17** owns around eight and fourfold increased inhibitory potency comparing to its glucosyl C2-epimer **13** on CDC25B and PTP1B, respectively. Moreover, the specificity of compound **17** toward CDC25B over other PTPs tested is also much enhanced than that of compound **13**. These data suggest that the click ligation of the threoninyl precursor with C6-modified monosaccharides may result in a similar inhibitory pattern toward PTPs. However, the PTP inhibitory effect of C6-triazoloserinyl glycosides is largely dependent on the epimeric sugar scaffolds whereon the serinyl precursor was introduced with the mannosyl derivative being the most potent.

The structurally varied C1-modified glucosides **19** and **20** bearing one additional benzyl group displayed generally improved inhibition on the tested PTPs compared with their C6-modified glycosyl analogs. Triazole-linked serinyl glucoside **19** possesses the best PTP1B and CDC25B inhibitory activities among this series with IC_{50} values equal to 6 μM. Moreover, this compound also showed enhanced IC_{50} values on TCPTP (13 μM), CDC25A (24 μM), SHP-1 (40 μM), and SHP-2 (47 μM). In contrast, despite the existence of the additional methyl group on the threoninyl residue of glucoside **20** may render its slightly decreased inhibitory potency toward PTP1B (IC_{50} = 7 μM) and CDC25B (IC_{50} = 12 μM), the selectivity of this compound has, however, been increased.

In an attempt to verify whether the benzyl groups on the identified PTPs inhibitors are crucial toward their corresponding activity, the debenzylated analog of the most potent inhibitor **19** was synthesized. As shown in Scheme 2, this click reaction was directly achieved from the known 1-azido glucoside **21**[19] and *O*-alkynyl Boc-L-serine **22**[20] under microwave irradiation at 60 °C for a ramp time of 5 min and a hold time of 15 min, affording the desired product **23** in a yield of 60%. This relatively low yield could presumably be ascribed to the partial conversion of the alkynoic acid **22** into enol lactones under the catalysis of Cu(I).[21] As shown in Table 1, the successive inhibitory assay showed that compound **23** is inactive on both PTP1B and CDC25B (IC_{50} >120 μM), which positively demonstrates that the benzyl groups appended with the sugar scaffold are significant for enhancing the binding affinity of the triazole-linked sugar-amino acid hybrids constructed.

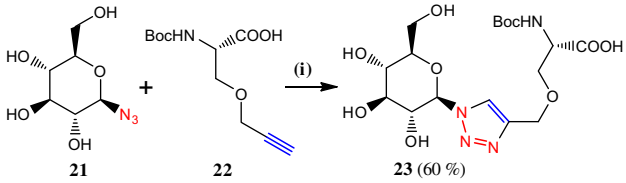
For evaluating the inhibitory modality of this compound series, the most active inhibitor **19** was selected for enzymatic kinetic study on PTP1B and CDC25B, respectively. The effect of compound **19** on PTP1B- and CDC25B-catalyzed reaction was studied at five different concentrations and the resulting *Eadie–Hofstee* plots[17] are given in Figure 2 (**19**-PTP1B) and 3 (**19**-CDC25B). Clearly, the diagrams in Figure 2 confirm that compound **19** is a competitive PTP1B inhibitor as the V_{max} value (Fig. 3B) retained invariable while the K_m value (Fig. 3A) increased with the increasing compound concentration. In contrast, the plots in Figure 3 are consistent with a mixed-type inhibition pattern of **19** on CDC25B due to the increasing K_m value (Fig. 4A) and concomitantly decreasing V_{max} value (Fig. 4B) upon the gradually increased compound concentration. The K_i value of the competitive PTP1B inhibitor **19** is assigned to 3.9 μM.

The MTT assay was then performed to preliminarily assess the cellular activity of the PTP inhibitors. As shown in Table 2, except the galactosyl serine **15** (IC_{50} >120 μM), all compounds showed micromolar activity on the selected human cancer cell lines including A549 (lung), Hela (cervix) and HCT-116 (colon). The glycosyl C6-triazoloserine and threonine **13**, **14**, and **16**–**18** are less toxic on A549 with the glucosyl threonine **14** and mannosyl threonine

Table 1
Inhibitory activity of compounds **13**–**20** on PTPs

Compd	IC_{50} (μM)[a,b,c,d]			
	PTP1B	CDC25B	TCPTP	CDC25A
13	67.8 ± 4.9	67.5 ± 7.5	>120	>120
14	22.3 ± 5.0	27.1 ± 5.0	80.7 ± 5.6	118.7 ± 16.1
15	20.9 ± 1.7	20.6 ± 7.1	54.9 ± 10.0	>120
16	15.3 ± 0.5	31.1 ± 6.6	>120	>120
17	18.5 ± 2.0	8.2 ± 3.8	89.2 ± 7.3	26.8 ± 25.1
18	26.0 ± 6.7	12.2 ± 4.8	105.3 ± 6.2	51.6 ± 10.4
19	5.9 ± 0.4	6.2 ± 3.0	12.5 ± 1.1	24.3 ± 1.5
20	7.1 ± 1.0	11.6 ± 1.8	38.6 ± 8.3	53.0 ± 1.8
23	>120	>120	n.d.[e]	n.d

[a] Except compound **19**, all other compounds herein showed no inhibitory activity on SHP-1, SHP-2 (IC_{50} >120 μM).
[b] The IC_{50} values of compound **19** on SHP-1 and SHP-2 are 40.4 ± 1.4 and 47.6 ± 8.9 μM, respectively.
[c] All compounds herein showed no inhibitory activity on LAR (IC_{50} >120 μM).
[d] Values are means of three experiments.
[e] n.d. = not determined.



Scheme 2. Reagents and conditions: (i) Na ascorbate, $CuSO_4 \cdot 5H_2O$, CH_2Cl_2/H_2O (1:1, v/v), microwave irradiation (60 °C).

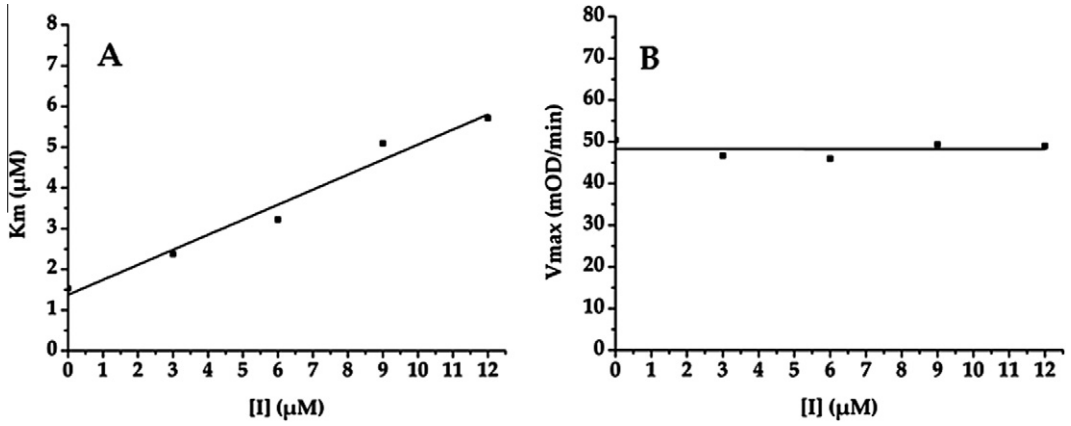


Figure 2. Inhibition of PTP1B catalyzed *p*NPP hydrolysis by compound **19**. Experiments were performed at 30 °C and pH 6.5.

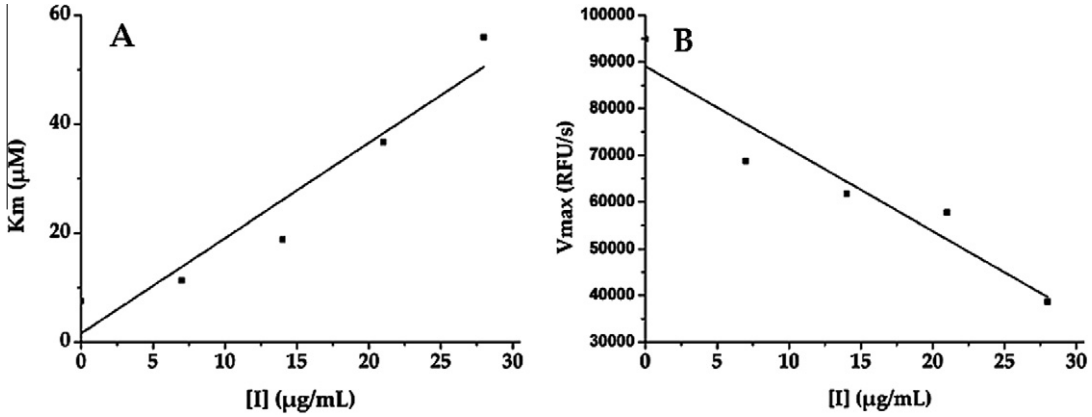


Figure 3. Inhibition of CDC25B-catalyzed *p*NPP hydrolysis by compound **19**. Experiments were performed at 30 °C and pH 6.5.

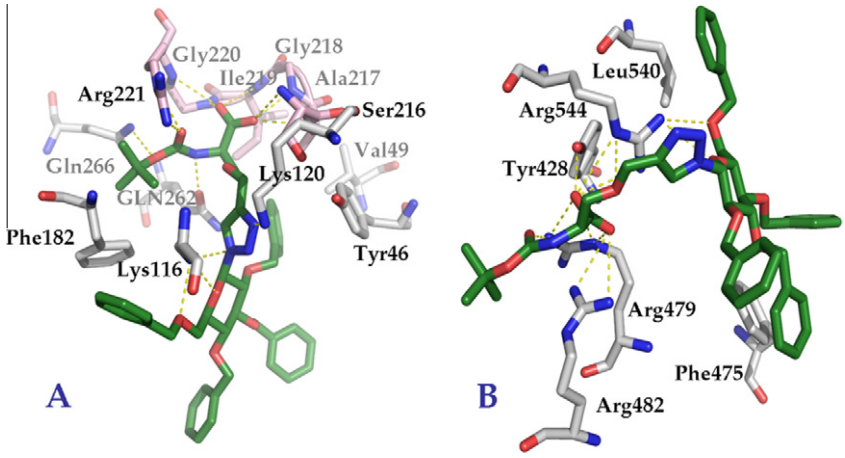


Figure 4. Plausible binding mode of compound **19** with (A) PTP1B and (B) CDC25B. The compound was shown as green stick and residues in PTP shown as light gray line. Nitrogen atoms are in blue and oxygen atoms in red. Hydrogen bonds were shown as yellow dashed lines and nonpolar interactions were not shown.

18 being around twofold more active than the rest. Their glucosyl C1-substituted analogs **19** and **20** exhibited similarly increased cytotoxicity.

The activities of the C6-modified glucosyl serine **13**, galactosyl threonine **16** and mannosyl serine **17** were almost twofold enhanced toward Hela, whereas those of the glucosyl threonine **14** and mannosyl threonine **18** remained unchanged. Likewise, the glucosyl C1-serine **19** and threonine **20** own enhanced cytotoxicity compared to their C6-modified analogs. Finally, these compounds showed further improved potency on HCT-116. Parallel IC_{50} values were observed among glycosyl C6-substituted derivatives with the glucosyl serine **13** being slightly less potent. However, their C1-substituted counterparts **19** and **20** further exhibited approximately threefold improved activity, demonstrating that these triazolyl sugar-amino acid hybrids are more specific toward HCT-116 inhibition.

Table 2
Cytotoxicity of compounds **13**–**20** on cancer cell lines

Compd	IC_{50} (μM)[a]		
	A549	Hela	HCT-116
13	119.5 ± 6.4	75.4 ± 12.4	46.3 ± 4.2
14	62.76 ± 4.8	69.5 ± 11.3	33.5 ± 2.1
15	>120	>120	>120
16	107.56 ± 11.6	56.4 ± 7.8	36.6 ± 3.1
17	97.3 ± 16.2	54.2 ± 5.3	32.3 ± 2.3
18	57.2 ± 3.1	53.7 ± 5.7	32.2 ± 3.0
19	35.2 ± 3.2	25.3 ± 2.4	12.8 ± 1.3
20	25.3 ± 3.4	31.5 ± 2.8	12.0 ± 1.2

[a] Values are means of three experiments.

2.3. Docking study

Eventually, the plausible complex of compound **19** with, respectively, PTP1B and CDC25B was tentatively proposed via docking simulation, illustrated in Figure 4. The most likely binding manner of the competitive inhibitor **19** with PTP1B is shown in Figure 4A. A densely functionalized hydrogen-bonding network is generated between the deeply inserted carboxylic acid precursor of the triazoloacid with amino acid residues including Ser216, Ala217, Gly218, Ile219, and Gly220 of the catalytic site cavity. This is similar to the reported co-crystallization of PTP1B with the structurally analogous isoxazole acids.[14]

In addition, the adjacent NHBoc group also made three hydrogen bonds with Arg221 and Gln266 while same interactions concurrently occurred between the oxygen atoms on glycosyl ring and C6-benzyl group with Lys116, and two nitrogen atoms on triazole ring with Lys116 and Lys120, respectively. Hydrophobic contacts were subsequently observed between the C2-distal benzene group with Tyr46 and Val49, and the C6-distal benzene moiety with Phe182 on WPD loop.

The plausible complex of the mixed-type inhibitor **19** with CDC25B was then elaborated in Figure 4B. Similarly, a series of hydrogen bonds were made between the carboxylic acid and the NHBoc group with Tyr428, Arg479, Arg482, and Arg544 in the catalytic area of CDC25B, while hydrogen-bonding interactions were also observed between the oxygen atoms of glucosyl ring and benzyl group with Arg544. Moreover, strong nonpolar contacts were generated between the C6-distal benzene group with Leu540 while a π–π stacking of C3-distal benzene with the benzene residue of Phe475 might contribute largely to the binding affinity.

Since there is no available crystal structure of triazolyl sugar-amino acid hybrids with either PTP1B or CDC25B, the above-depicted binding modes are preliminary whereas further crystallographic evidence is desirable.

3. Conclusion

In summary, we have shown in this study that the facilely 'clicked' triazolyl sugar-amino acid hybrids could serve as competent PTP1B and CDC25B inhibitors with micromole-ranged inhibitory activity. The introduction of triazolo-serinyl or threoninyl precursors onto different monosaccharide templates may result in distinct inhibitory potency and selectivity of the constructed products. Furthermore, MTT assay identified these compounds are toxic toward several cancer cell lines with specificity toward HCT-116, indicating their potential value of being active on cellular level. We hence consider that the modular 'clicking' of natural amino acids onto structurally and configurationally diverse sugar templates could probably represent a concise strategy toward the efficient acquisition of new promising PTP inhibitor entities in a productive way.

4. Experimental section

All purchased chemicals and reagents are of high commercially available grade. Solvents were purified by standard procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400 MHz spectrometer in $CDCl_3$, D_2O or DMSO-d_6 solutions. Optical rotations were measured using a Perkin–Elmer 241 polarimeter at room temperature. Analytical thin-layer chromatography was performed on E. Merck aluminum percolated plates of Silica Gel 60F-254 with detection by UV and by spraying with 6 N H_2SO_4 and heating at 300 °C. The microwave-assisted reaction was performed in a Yalian (YL8023B1) system. High resolution mass spectra (HRMS) were recorded on an MA1212 instrument using standard conditions (ESI, 70 eV).

4.1. General procedure for the conventional click reaction

To a biphasic solution of the azide (1 equiv) and the alkyne (1 equiv) in CH_2Cl_2 (8 mL) and water (8 mL), sodium ascorbate (6 equiv) and $CuSO_4{\cdot}5H_2O$ (3 equiv) were added. This mixture was stirred vigorously at rt for 8 h and was then directly diluted with CH_2Cl_2, washed with brine and dried over $MgSO_4$. The combined organic layer was filtered and concentrated in vacuum to give a crude residue which was then purified by column chromatography.

4.1.1. (*R*)-Benzyl-2-((*tert*-butoxycarbonyl)amino)-3-((1-(((2*R*,3*R*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)propanoate (5)

Compound **1** (226 mg, 0.5 mmol) was treated with **a** (169 mg, 0.5 mmol) according to the general procedure. Purification by chromatography (EtOAc/Petroleum ether; 1:10–1:2) gave 341 mg (90%) of **5** as a colorless oil. R_f = 0.6 (EtOAc/Petroleum ether; 1:1). $[\alpha]_D$ = −3.7 (*c* 0.2, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41 (s, 1H), 7.36–7.26 (m, 20H), 5.37 (d, *J* = 7.6 Hz, 1H), 5.23 (d, *J* = 12.4 Hz, 1H), 5.11 (d, *J* = 12.4 Hz, 1H), 4.98 (d, *J* = 10.8 Hz, 1H), 4.91 (d, *J* = 10.8 Hz, 1H), 4.81 (d, *J* = 10.8 Hz, 1H), 4.74 (t, *J* = 12.4 Hz, 2H), 4.63–4.29 (m, 8H), 4.00 (t, *J* = 9.2, Hz, 1H), 3.92 (br s, 2H), 3.72 (dd, *J* = 2.4, 9.2 Hz, 1H), 3.43 (dd, *J* = 3.6, 10.0 Hz, 1H), 3.16 (d, *J* = 3.6 Hz, 3H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 155.5, 144.2, 138.5, 138.0, 135.6, 128.5, 128.4, 128.2, 128.0, 127.9, 127.7, 124.1, 99.7, 81.8, 80.0, 79.9, 78.0, 75.7, 74.9, 73.3, 70.2, 69.1, 66.9, 64.8, 55.3, 54.1, 50.6, 28.3; HRMS (*m*/*z*): calcd for $C_{46}H_{54}N_4O_{10}$+H: 823.3918, found: 823.3918.

4.1.2. (2*S*,3*S*)-Benzyl-2-((*tert*-butoxycarbonyl)amino)-3-((1-(((2*R*,3*R*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy) butanoate (6)

Compound **1** (379 mg, 0.8 mmol) was treated with **b** (268 mg, 0.8 mmol) according to the general procedure. Purification by chromatography (EtOAc/Petroleum ether; 1:10–1:2) gave 528 mg (80%) of **6** as a colorless oil. R_f = 0.6 (EtOAc/Petroleum ether; 1:1). $[\alpha]_D$ = −0.07 (*c* 0.1, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35–7.29 (m, 21H), 5.23 (d, *J* = 9.2 Hz, 1H), 5.17 (d, *J* = 12.4 Hz, 1H), 5.07 (d, *J* = 12.4 Hz, 1H), 4.98 (d, *J* = 10.4 Hz, 1H), 4.90 (d, *J* = 10.8 Hz, 1H), 4.80 (d, *J* = 10.8 Hz, 1H), 4.73 (t, *J* = 12.4 Hz, 2H), 4.62–4.32 (m, 8H), 4.15 (d, *J* = 6.0, Hz, 1H), 4.00 (t, *J* = 9.2, Hz, 1H), 3.91 (t, *J* = 7.2, Hz, 1H), 3.43 (dd, *J* = 3.2, 10.6 Hz, 1H), 3.17 (s, 3H), 1.44 (s, 9H), 1.23 (d, *J* = 8.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.8, 156.1, 144.7, 138.3, 138.0, 135.5, 128.5, 128.2, 128.0, 127.7, 124.0, 98.0, 81.8, 79.9, 78.0, 75.7, 74.9, 73.4, 69.1, 67.0, 62.6, 58.4, 55.3, 51.6, 28.3, 16.3; HRMS (*m*/*z*): calcd for $C_{46}H_{54}N_4O_{10}$+H: 823.3918, found: 823.4067.

4.1.3. (*R*)-Benzyl-2-((*tert*-butoxycarbonyl)amino)-3-((1-(((2*R*,3*S*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy) propanoate (7)

Compound **2** (142 mg, 0.3 mmol) was treated with **a** (116 mg, 0.3 mmol) according to the general procedure. Purification by chromatography (EtOAc/Petroleum ether; 1:10–1:2) gave 202 mg (84%) of **7** as a yellow oil. R_f = 0.6 (EtOAc/Petroleum ether; 1:1). $[\alpha]_D$ = +0.5 (*c* 0.3, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.43–7.29 (m, 21H), 5.34 (d, *J* = 12.0 Hz, 1H), 5.23 (d, *J* = 12.4 Hz, 1H), 4.91 (d, *J* = 11.6 Hz, 2H), 4.85 (d, *J* = 12.0 Hz, 1H), 4.77–4.45 (m, 8H), 4.33–4.12 (m, 2H), 4.04 (dd, *J* = 3.6, 10.0 Hz, 1H), 4.00–3.81 (m, 4H), 3.69 (dd, *J* = 2.8, 9.2 Hz, 1H), 2.97 (s, 3H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 155.5, 144.1, 138.5, 138.3, 138.0, 135.5, 128.7, 128.6, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 124.1, 98.7, 80.0, 79.2, 78.8, 76.2, 75.1, 74.7, 74.4, 73.9, 73.7, 70.3, 69.5, 67.1, 64.7, 57.1, 55.2, 54.1, 51.2, 28.3; HRMS (*m*/*z*): calcd for $C_{46}H_{54}N_4O_{10}$+H: 823.3918, found: 823.3909.

4.1.4. (2*S*,3*S*)-Benzyl-2-((*tert*-butoxycarbonyl)amino)-3-((1-(((2*R*,3*S*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy) butanoate (8)

Compound **2** (179 mg, 0.4 mmol) was treated with **b** (129 mg, 0.4 mmol) according to the general procedure. Purification by chromatography (EtOAc/Petroleum ether; 1:10–1:2) gave 314 mg (85%) of **8** as a white powder. R_f = 0.6 (EtOAc/Petroleum ether; 1:1). $[\alpha]_D$ = −7.2 (*c* 0.1, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.29 (m, 21H), 5.14 (d, *J* = 12.4 Hz, 2H), 5.07 (d, *J* = 6.0 Hz, 1H), 5.04 (d, *J* = 5.2 Hz, 1H), 4.90 (d, *J* = 11.6 Hz, 1H), 4.84 (d, *J* = 12.0 Hz, 1H), 4.75 (d, *J* = 11.6 Hz, 1H), 4.70–4.59 (m, 4H), 4.31 (d, *J* = 9.6 Hz, 1H), 4.14 (d, *J* = 11.2 Hz, 2H), 4.03 (dd, *J* = 3.6, 10.0 Hz, 1H), 3.98 (br s, 1H), 3.92 (dd, *J* = 2.8, 10.0 Hz, 1H), 3.83 (d, *J* = 2.0 Hz, 1H), 2.98 (s, 3H), 1.44 (s, 9H), 1.24 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.8, 156.1, 144.5, 138.2, 138.1, 135.6, 128.6, 128.5, 128.3, 128.2, 128.0, 127.7, 126.1, 125.8, 124.2, 99.2, 80.2, 79.8, 75.1, 75.0, 74.7, 74.6, 73.0, 72.1, 70.7, 67.0, 62.5, 60.3, 58.5, 54.9, 51.0, 28.3, 16.3; HRMS (*m*/*z*): calcd for $C_{47}H_{56}N_4O_{10}$+Na: 859.3894, found: 859.3898.

4.1.5. 2-(*tert*-Butoxycarbonylamino)-3-((1-(((2*R*,3*R*,4*S*,5*S*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)propanoate benzyl ether (9)

Compound **3** (483.4 mg, 1.0 mmol) was treated with **a** (316 mg, 1.0 mmol) according to the general procedure. Purification by chromatography (EtOAc/Petroleum ether; 1:10–1:2) gave 646 mg (87%) of **9** as a colorless oil. R_f = 0.6 (EtOAc/Petroleum ether; 1:1). $[\alpha]_D$ = +0.05 (*c* 0.1, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.59 (s, 1H), 7.37–7.32 (m, 20H), 5.36 (d, *J* = 8.0 Hz, 1H), 5.23 (d, *J* = 12.4 Hz, 1H), 5.12 (d, *J* = 12.4 Hz, 1H), 4.95 (d, *J* = 11.2 Hz, 1H), 4.75 (d, *J* = 12.0 Hz, 1H), 4.69 (d, *J* = 11.6 Hz, 1H), 4.67–4.45 (m, 9H), 3.92 (dd, *J* = 6.4, 8.8 Hz, 2H), 3.90–3.87 (m, 1H), 3.78 (br s, 1H), 3.71–3.62 (m, 2H), 3.08 (d, *J* = 6.0 Hz, 3H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 155.5, 144.0, 138.2, 138.1, 135.5, 128.6, 128.5, 128.4, 128.2, 127.8, 127.7, 124.3, 99.2, 80.2, 80.0, 75.1, 75.0, 74.9, 74.6, 73.0, 72.9, 72.1, 70.6, 70.5, 70.1, 67.1, 64.8, 64.7, 64.8, 54.1, 51.0, 29.7, 29.6, 28.3; HRMS (*m*/*z*): calcd for $C_{46}H_{54}N_4O_{10}$+H: 823.3918, found: 823.3922.

4.1.6. (2*S*,3*S*)-Benzyl 2-((*tert*-butoxycarbonyl)amino)-3-((1-(((2*R*,3*R*,4*S*,5*S*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy) butanoate (10)

Compound **3** (586 mg, 1.2 mmol) was treated with **b** (421 mg, 1.2 mmol) according to the general procedure. Purification by chromatography (EtOAc/Petroleum ether; 1:8–1:1) gave 868 mg (87%) of **10** as a colorless oil. R_f = 0.5 (EtOAc/Petroleum ether; 1:1). $[\alpha]_D$ = −0.05 (*c* 0.2, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.48 (s, 1H), 7.36–7.26 (m, 20H), 5.24 (d, *J* = 9.6 Hz, 1H), 5.16 (d, *J* = 11.6 Hz, 1H), 5.10 (d, *J* = 12.4 Hz, 1H), 4.96 (d, *J* = 11.2 Hz, 1H), 4.74 (d, *J* = 12.0 Hz, 1H), 4.68 (d, *J* = 12.4 Hz, 1H), 4.67–4.56 (m, 6H), 4.40–4.35 (m, 2H), 4.32 (dd, *J* = 6.0, 9.2 Hz, 1H), 4.18–4.10 (m, 1H), 3.92–3.85 (m, 2H), 3.78 (t, *J* = 2.0 Hz, 1H), 3.66 (t, *J* = 9.6 Hz, 1H), 3.07 (s, 3H), 1.45 (s, 9H), 1.22 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.8, 156.1, 144.5, 138.2, 138.1, 135.6, 128.6, 128.5, 128.3, 128.2, 127.8, 127.7, 126.1, 125.8, 124.2, 99.2, 80.2, 79.8, 75.1, 75.0, 74.7, 74.6, 73.0, 72.1, 70.7, 67.0, 62.5, 60.3, 58.5, 54.9, 51.0, 28.3, 16.3; HRMS (*m*/*z*): calcd for $C_{47}H_{56}N_4O_{10}$+Na: 859.3894, found: 859.3901.

4.1.7. (*R*)-Benzyl-2-((*tert*-butoxycarbonyl)amino)-3-((1-((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl) tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)methoxy) propanoate (11)

Compound **4** (790 mg, 1.4 mmol) was treated with **a** (431 mg, 1.3 mmol) according to the general procedure. Purification by chromatography (EtOAc/Petroleum ether; 1:10–1:2) gave 899 mg (87%) of **11** as a colorless oil. R_f = 0.7 (EtOAc/Petroleum ether; 1:1). $[\alpha]_D$ = −1.9 (*c* 0.2, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.42 (s, 1H), 7.36–7.23 (m, 18H), 7.19–7.15 (m, 5H), 6.94–6.92 (m, 2H), 5.53 (d, *J* = 9.2 Hz, 1H), 5.40 (d, *J* = 8.8 Hz, 1H), 5.21 (d, *J* = 12.4 Hz, 1H), 5.06 (d, *J* = 12.4 Hz, 1H), 4.92 (t, *J* = 11.2 Hz, 2H), 4.86 (d, *J* = 10.4 Hz, 1H), 4.62–4.44 (m, 8H), 4.07 (d, *J* = 10.4 Hz, 1H), 4.04–4.00 (m, 1H), 3.86–3.79 (m, 2H), 3.75–3.68 (m, 4H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.5, 155.5, 144.7, 138.2, 137.7, 137.0, 135.5, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 122.0, 87.6, 85.5, 80.6, 80.1, 78.0, 75.8, 75.3, 74.9, 73.6, 70.6, 68.0, 67.1, 65.0, 54.1, 28.4; HRMS (*m*/*z*): calcd for $C_{52}H_{59}N_4O_{10}$+H: 899.4231, found: 899.4205.

4.1.8. (2*S*,3*S*)-Benzyl 2-((*tert*-butoxycarbonyl)amino)-3-((1-((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl) tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)methoxy) butanoate (12)

Compound **4** (623 mg, 1.1 mmol) was treated with **b** (320 mg, 0.9 mmol) according to the general procedure. Purification by chromatography (EtOAc/Petroleum ether; 1:8–1:2) gave 512 mg (62%) of **12** as a colorless oil. R_f = 0.3 (EtOAc/Petroleum ether; 1:4). $[\alpha]_D$ = −3.4 (*c* 0.1, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.25 (m, 21H), 7.20–7.17 (m, 3H), 6.94–6.90 (m, 2H), 5.51 (d, *J* = 9.2 Hz, 1H), 5.23 (d, *J* = 10.0 Hz, 1H), 5.15 (d, *J* = 12.4 Hz, 1H), 5.03 (d, *J* = 12.0 Hz, 1H), 4.95–4.89 (m, 2H), 4.86 (d, *J* = 10.4 Hz, 1H), 4.61–4.32 (m, 7H), 4.08 (d, *J* = 10.8 Hz, 1H), 4.00 (t, *J* = 9.2 Hz, 1H), 3.86–3.79 (m, 2H), 3.74–3.66 (m, 4H), 1.44 (s, 9H), 1.25 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9, 156.2, 145.3, 138.2, 137.7, 137.0, 135.5, 128.6, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 126.0, 125.8, 121.8, 87.5, 85.5, 80.6, 80.0, 78.0, 75.8, 75.3, 75.2, 74.8, 73.5, 68.0, 67.1, 62.7, 60.4, 58.3, 28.4, 16.2; HRMS (*m*/*z*): calcd for $C_{53}H_{60}N_4O_{10}$+Na: 913.4388, found: 913.4393.

4.2. Preparation of (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-((1-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)methoxy) propanoic acid (23)

To a well-stirred biphasic solution of sugar azide **21** (226 mg, 1.1 mmol) and alkynyl amino acid **22** (205 mg, 1.0 mmol) in *t*BuOH/THF/H_2O (8/8/8 mL), sodium ascorbate (0.2 equiv) and $CuSO_4{\cdot}5H_2O$ (0.1 equiv) was added. Then the mixture was transferred to the microwave oven at 60 °C for a ramp time of 5 min

and hold time of 15 min. Upon completion, the resulting mixture was diluted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, filtered and evaporated to give a crude residue. Purification by chromatography (EtOAc/EtOH; 1:8–1:4) gave 267 mg (60%) of **23** as a white powder. R_f = 0.5 (EtOAc/CH_3OH; 10:1). $[\alpha]_D$ = −10.9 (*c* 0.1, CH_3OH); ^1H NMR (400 MHz, D_2O): δ = 8.21 (s, 1H), 5.71 (d, *J* = 9.2 Hz, 1H), 4.71–4.64 (m, 2H), 4.32 (br s, 1H), 3.96 (t, *J* = 9.2 Hz, 1H), 3.87 (d, *J* = 11.2 Hz, 2H), 3.80 (dd, *J* = 3.6, 10.4 Hz, 1H), 3.76 (d, *J* = 5.2 Hz, 1H), 3.37–3.66 (m, 3H), 3.59 (t, *J* = 9.2 Hz, 1H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, D_2O): δ = 176.6, 173.8, 160.0, 146.5, 126.8, 89.9, 84.0, 81.3, 78.4, 74.8, 70.1, 65.8, 62.9, 59.9, 56.7, 56.5, 32.1, 30.1, 19.3; HRMS (*m*/*z*): calcd for $C_{17}H_{29}N_4O_{10}$−H: 447.1727, found: 447.1735.

4.3. General procedure for the debenzylation

To a solution of benzyl ester in MeOH (15 mL), was added 10% Pd/C (5 wt %). The mixture was stirred vigorously under hydrogen atmosphere for 20 min. Then the mixture was filtered and concentrated in vacuum to afford the unique product without further purification.

4.3.1. (*R*)-2-((*tert*-Butoxycarbonyl)amino)-3-((1-(((2*R*,3*R*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy) propanoic acid (13)

From compound **5** (121 mg, 0.2 mmol), afforded **13** as a white powder (92 mg, 85%). R_f = 0.7 (EtOAc/EtOH; 8:1). $[\alpha]_D$ = −15.7 (*c* 0.1, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6): δ = 1.38 (s, 9H), 3.04 (s, 3H), 3.35–3.30 (m, 3H), 3.48–3.43 (m, 3H), 3.63 (d, *J* = 5.2, 3H), 3.80 (t, *J* = 9.6 Hz, 1H), 3.87 (t, *J* = 8.0 Hz, 1H), 4.08 (br s, 1H), 4.63–4.49 (m, 3H), 4.88–4.78 (m, 2H), 6.78 (d, *J* = 7.6 Hz, 1H), 7.35–7.30 (m, 15H), 8.03 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 28.1, 50.3, 53.8, 54.3, 63.5, 68.8, 69.0, 71.4, 74.0, 74.5, 78.2, 78.4, 79.4, 81.0, 96.7, 125.0, 127.4, 127.5, 127.6, 127.8, 128.1, 128.2, 128.3, 138.1, 138.3, 138.5, 143.5, 155.3, 171.9; HRMS (*m*/*z*): calcd for $C_{39}H_{48}N_4O_{10}$+H: 733.3449, found: 733.3446.

4.3.2. (2*S*,3*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-((1-(((2*R*,3*R*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)butanoic acid (14)

From compound **6** (111 mg, 0.1 mmol) afforded **14** as a white powder (98 mg, 99%). R_f = 0.7 (EtOAc/EtOH; 8:1). $[\alpha]_D$ = −11.7 (*c* 0.1, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6): δ = 7.99 (s, 1H), 7.38–7.26 (m, 15H), 6.31 (d, *J* = 8.4 Hz, 1H), 4.89–4.78 (m, 2H), 4.64–4.47 (m, 3H), 3.99 (d, *J* = 6.8 Hz, 2H), 3.89–3.84 (m, 1H), 3.80 (t, *J* = 9.2 Hz, 1H), 3.48–3.42 (m, 5H), 3.35–3.30 (m, 5H), 3.05 (s, 3H), 1.39 (s, 9H), 1.07 (dd, *J* = 6.0, 12.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.1, 155.7, 144.1, 138.5, 138.3, 138.1, 128.3, 128.2, 128.1, 127.8, 127.6, 127.5, 127.4, 124.8, 96.7, 81.0, 79.4, 78.3, 74.5, 74.0, 73.9, 71.4, 68.8, 61.7, 58.0, 56.0, 54.4, 50.3, 28.1, 16.4; HRMS (*m*/*z*): calcd for $C_{40}H_{51}N_4O_{10}$+H: 747.3605, found: 747.3608.

4.3.3. (*R*)-2-((*tert*-Butoxycarbonyl)amino)-3-((1-(((2*R*,3*S*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy) propanoic acid (15)

From compound **7** (105 mg, 0.1 mmol) afforded **15** as a white powder (70 mg, 81%). R_f = 0.6 (EtOAc/EtOH; 8:1). $[\alpha]_D$ = −3.3 (*c* 0.2, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6): δ = 8.04 (s, 1H), 7.41–7.28 (m, 15H), 6.66 (d, *J* = 6.8 Hz, 1H), 4.89 (d, *J* = 11.2, 1H), 4.78 (t, *J* = 12.0 Hz, 1H), 4.66–4.61 (m, 1H), 4.51–4.46 (m, 3H), 4.15 (s, 1H), 4.07–4.02 (m, 3H), 3.90–3.86 (m, 2H), 3.69–3.60 (m, 3H), 3.52–3.34 (m, 5H), 2.91 (s, 3H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 171.9, 155.3, 143.4, 138.7, 138.6, 138.5, 128.2, 128.1, 128.0, 127.6, 127.5, 127.4, 127.3, 124.8, 97.5, 78.2, 77.7, 75.3, 74.9, 74.0, 71.6, 69.2, 69.0, 63.4, 54.2, 53.9, 50.5, 28.1; HRMS (*m*/*z*): calcd for $C_{39}H_{49}N_4O_{10}$+H: 733.3449, found: 733.3451.

4.3.4. (2*S*,3*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-((1-(((2*R*,3*S*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)butanoic acid (16)

From compound **8** (113 mg, 0.1 mmol) afforded **16** as a white powder (101 mg, 92%). R_f = 0.7 (EtOAc/EtOH; 8:1). $[\alpha]_D$ = −0.3 (*c* 0.1, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6): δ = 8.00 (s, 1H), 7.41–7.29 (m, 15H), 6.26 (d, *J* = 8.4 Hz, 1H), 4.90 (d, *J* = 11.2 Hz, 1H), 4.78 (t, *J* = 12.0, 1H), 4.66–4.62 (m, 1H), 4.58–4.43 (m, 3H), 4.15 (s, 1H), 4.07 (dd, *J* = 3.2, 9.6 Hz, 1H), 3.98 (d, *J* = 8.0, 2H), 3.91–3.84 (m, 3H), 3.53 (dd, *J* = 8.0, 9.6 Hz, 1H), 3.45 (dd, *J* = 6.8, 14.0 Hz, 1H), 3.33 (br s, 4H), 2.93 (s, 3H), 1.39 (s, 9H), 1.09 (d, *J* = 5.6, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.1, 155.6, 144.1, 138.7, 138.6, 128.2, 128.1, 127.5, 127.4, 127.3, 124.5, 97.6, 78.3, 77.8, 75.5, 75.0, 74.0, 71.6, 69.2, 61.8, 58.0, 54.3, 50.5, 28.1, 16.4; HRMS (*m*/*z*): calcd for $C_{40}H_{51}N_4O_{10}$+H: 747.3605, found: 747.3608.

4.3.5. (*R*)-2-((*tert*-Butoxycarbonyl)amino)-3-((1-(((2*R*,3*R*,4*S*,5*S*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy) propanoic acid (17)

From compound **9** (134 mg, 0.2 mmol) afforded **17** as a white powder (111 mg, 93%). R_f = 0.7 (EtOAc/EtOH; 8:1). $[\alpha]_D$ = −5.9 (*c* 0.2, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6): δ = 8.06 (s, 1H), 7.39–7.23 (m, 15H), 6.83 (d, *J* = 8.0 Hz, 1H), 4.87 (d, *J* = 11.2 Hz, 1H), 4.78 (t, *J* = 12.0 Hz, 1H), 4.64–4.50 (m, 3H), 4.13–4.09 (m, 1H), 3.89 (t, *J* = 2.8 Hz, 1H), 3.84–3.76 (m, 2H), 3.63–3.58 (m, 4H), 3.43 (dd, *J* = 6.8, 14.0 Hz, 2H), 3.33 (br s, 5H), 3.01 (s, 3H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 171.9, 155.3, 143.4, 138.3, 138.2, 128.2, 128.1, 127.6, 127.5, 125.1, 98.0, 79.0, 78.2, 75.0, 74.1, 74.0, 71.9, 70.4, 70.0, 69.0, 63.5, 56.1, 54.1, 53.8, 50.4, 28.1, 27.8; HRMS (*m*/*z*): calcd for $C_{39}H_{49}N_4O_{10}$+H: 733.3449, found: 733.3453.

4.3.6. (2*S*,3*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-((1-(((2*R*,3*R*,4*S*,5*S*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)butanoic acid (18)

From compound **10** (128 mg, 0.2 mmol) afforded **18** as a white powder (105 mg, 92%). R_f = 0.5 (EtOAc/EtOH; 6:1). $[\alpha]_D$ = −0.6 (*c* 0.1, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6): δ = 8.00 (s, 1H), 7.41–7.29 (m, 15H), 6.26 (d, *J* = 8.4 Hz, 1H), 4.86 (d, *J* = 11.2 Hz, 1H), 4.78 (s, 1H), 3.88 (s, 1H), 4.68 (d, *J* = 10.8 Hz, 1H), 4.64 (t, *J* = 5.2, 3H), 4.57–4.51 (m, 3H), 4.44 (d, *J* = 12.4 Hz, 1H), 3.98 (d, *J* = 6.4 Hz, 2H), 3.83–3.75 (m, 2H), 3.62–3.37 (m, 4H), 3.02 (s, 3H), 1.38 (s, 9H), 1.08 (d, *J* = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.2, 155.7, 144.1, 138.3, 138.2, 128.2, 128.1, 127.8, 127.6, 127.5, 124.8, 98.0, 80.0, 78.3, 75.0, 74.0, 73.9, 71.8, 70.4, 70, 61.7, 58.1, 54.2, 50.4, 28.1, 16.4; HRMS (*m*/*z*): calcd for $C_{40}H_{51}N_4O_{10}$+H: 747.3605, found: 747.3608.

4.3.7. (*R*)-2-((*tert*-Butoxycarbonyl)amino)-3-((1-((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-((benzyloxy) methyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)propanoic acid (19)

From compound **11** (166 mg, 0.2 mmol) afforded **19** as a white powder (139 mg, 93%). R_f = 0.8 (EtOAc/EtOH; 6:1). $[\alpha]_D$ = −0.7 (*c* 0.6, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6): δ = 8.49 (s, 1H), 7.32–7.30 (m, 13H), 7.21–7.19 (m, 5H), 6.92 (d, *J* = 8.0 Hz, 1H), 6.87 (d, *J* = 2.8 Hz, 2H), 5.92 (d, *J* = 9.2 Hz, 1H), 4.85 (s, 2H), 4.77

(d, J = 10.8 Hz, 1H), 4.61–4.56 (m, 3H), 4.52–4.43 (m, 3H), 4.17 (t, J = 9.2 Hz, 2H), 4.00–3.91 (m, 3H), 3.71–3.49 (m, 7H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.2, 155.7, 144.1, 138.3, 138.2, 128.2, 128.1, 127.8, 127.6, 127.5, 124.8, 98.0, 80.0, 78.3, 75.0, 74.0, 73.9, 71.8, 70.4, 70.0, 61.7, 58.1, 54.2, 50.4, 28.1; HRMS (m/z): calcd for $C_{45}H_{53}N_4O_{10}$+H: 809.3762, found: 809.3765.

4.3.8. (2*S*,3*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-((1-((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)butanoic acid (20)

From compound **12** (125 mg, 0.1 mmol) afforded **20** as a white powder (106 mg, 94%). R_f = 0.6 (EtOAc/EtOH; 6:1). $[\alpha]_D$ = −0.6 (c 0.2, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.00 (s, 1H), 7.34–7.27 (m, 20H), 6.45 (d, J = 9.2 Hz, 1H), 4.86 (d, J = 11.6 Hz, 1H), 4.82 (d, J = 10.8 Hz, 1H), 4.77 (d, J = 3.2 Hz, 1H), 4.73–4.69 (m, 2H), 4.66–4.58 (m, 3H), 4.55–4.46 (m, 3H), 4.03–3.94 (m, 2H), 3.88–3.83 (m, 1H), 3.79 (t, J = 9.2, Hz, 1H), 3.45 (dd, J = 3.6, 9.6 Hz, 1H), 3.04 (s, 3H), 1.35 (s, 9H), 1.10 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.1, 155.8, 144.2, 138.3, 128.3, 128.2, 128.1, 127.8, 127.6, 127.5, 124.8, 96.7, 81.0, 79.4, 78.4, 78.3, 74.5, 74.0, 71.4, 68.8, 61.8, 58.1, 54.4, 50.3, 28.1, 16.5.

4.4. Inhibitory assay

The recombinant human PTP1B catalytic domain was expressed and purified according to procedures described previously.[17a] Enzymatic activity of PTP1B was determined at 30 °C by monitoring the hydrolysis of *p*NPP. Dephosphorylation of *p*NPP generates product *p*NP, which can be monitored at 405 nm. In a typical 100 μL assay, mixture containing 50 mM MOPS, pH 6.5, 2 mM *p*NPP and recombinant enzymes, PTP1B activities were continuously monitored on a SpectraMax 340 microplate reader at 405 nm for 2 min at 30 °C and the initial rate of the hydrolysis was determined using the early linear region of the enzymatic reaction kinetic curve. For calculating IC_{50}, inhibition assays were performed with 30 nM recombinant enzyme, 2 mM *p*NPP in 50 mM MOPS at pH 6.5, and the inhibitors diluted around the estimated IC_{50} values. IC_{50} was calculated from the nonlinear curve fitting of percent inhibition (inhibition (%)) versus inhibitor concentration [I] by using the following equation: inhibition (%) = $100/\{1 + (IC_{50}/[I])k\}$, where k is the Hill coefficient. To study the inhibition on other PTP family members, human CDC25A, CDC25B, TCPTP, SHP-1, SHP-2, and LARD1 were prepared and assays were performed according to procedures described previously.[17b]

4.5. Kinetic study

To determine inhibitor modality on PTP1B, assay was carried out in a 100 μL assay mixture contained 50 mM MOPS at pH 6.5, 30 nM PTP1B, *p*NPP in twofold dilution up to 80 mM, and different concentrations of inhibitor **19**. To determine inhibitor modality on CDC25B, assay was carried out in a 100 μL assay mixture contained 50 mM Tris–HCl, pH 8.0, 50 mM NaCl, 100 nM CDC25B, OMFP in twofold dilution up to 160 μM, 1 mM DTT, 1 mM EDTA, 1% glycerin, and **19** diluted around the estimated IC_{50} values. In the presence of the inhibitor, the *Michaelis–Menten* equation is described as $1/v = [K_m/(V_{max}\cdot[S])](1 + [I]/K_i) + 1/V_{max}$, where v is the initial rate, V_{max} is the maximum rate, and [S] is the substrate concentration. K_i value was obtained by linear re-plot of apparent K_m/V_{max} (slope) from primary reciprocal plot versus inhibitor concentration [I] according to the equation $K_m/V_{max} = 1 + [I]/K_i$.

4.6. MTT assay

Cancer cell lines were seeded onto a 96-well plate at a concentration of 2000 cells/well and incubated at 37 °C in 5% CO_2 for 24 h. A range of concentrations of the test compounds were added and the plate was incubated at 37 °C for 72 h before 40 μL MTT (5 mg/mL)/well was added. After 3 h incubation, the medium was removed and 100 μL DMSO was added to each well. The absorbance was measured on SpectraMax 340 microplate reader at 550 nm with a reference at 690 nm. The optical density of the result in MTT assay was directly proportional to the number of viable cells.

4.7. Docking simulation

The docking simulation was initiated with a crystal structure in complex with a reference ligand (for PTP1B, PDB code: 3EB1[9b] and for CDC25B, PDB code: 1QB05c). Water was removed from the original structure and the rest protein was prepared using the Protein preparation wizard (Schrödinger, LLC, New York, NY, 2005). Then compound **19** was docked to the active site of the protein using the Induced Fit Docking workflow (Schrödinger, LLC, New York, NY, 2005). The center atom was set to be a virtual center of referenced key residues: Phe182, Cys215 and Gly259 (PTP1B), and Cys473, Glu474, Glu478, and Met531 (CDC25B).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.05.049.

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