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Discovery of potent, selective and orally bioavailable triaryl-sulfonamide based PTP1B inhibitors ☆

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ABSTRACT

A novel series of *p*Tyr mimetics containing triaryl-sulfonamide derivatives (**5a**–**r**) are reported as potent and selective PTP1B inhibitors. Some of the test compounds (**5o** and **5p**) showed excellent selectivity towards PTP1B over various PTPs, including TCPTP (in vitro). The lead compound **5o** showed potent anti-diabetic activity (in vivo), along with improved pharmacokinetic profile. These preliminary results confirm discovery of highly potent and selective PTP1B inhibitors for the treatment of T2DM.

Over the past decade, there has been an alarming increase in the metabolic syndrome such as obesity and diabetes.[1,2] Patients suffer from obesity-induced type 2 diabetes (diabesity) are at increased risk of cardiovascular diseases and pose huge economic burden on healthcare services.[3] Clinically type 2 diabetes mellitus (T2DM) is characterized by persistent hyperglycemia, either due to defects in insulin secretion, insulin resistance or both.[4] Currently diabetic patients are treated with various oral antihyperglycemic agents; however, over a period of time, T2DM subjects lose their response to these agents and thereby require insulin therapy. Except incretin therapies, most of the available antihyperglycemic agents, including insulin promote weight gain, which further aggravates obesity associated cardiovascular risk and insulin resistance.[5–9] Thus, there is an urgent need to develop novel agents for glycemic control.

Protein tyrosine phosphatase 1B (PTP1B) enzyme acts as a negative regulator in insulin signaling pathways. Inhibition of PTP1B enzyme activity exhibits potential for enhancing insulin action by prolonging the phosphorylated state of the insulin receptor.[10] Several structurally diverse small-molecule based PTP1B inhibitors have been developed.[11] Initially, PTP1B inhibitors were designed to bind to the active site (Site-1/A).[12] However, achieving PTP1B selectivity over closely associated PTPs (PTPα, LAR, CD45, VHR, SHP-1, SHP-2 and T-cell protein tyrosine phosphatase (TCPTP)) is one of the major challenge, as the closely associated PTPs share a high degree of sequence homology (92%).[13] Lack of oral bioavailability is another aspect in the development of potent and selective PTP1B inhibitors, as the majority of the active site directed PTP1B inhibitors exhibit limited cell permeability.[14,15]

An additional non-catalytic aryl-phosphate binding site (site-2/B) was also identified, proximal to the catalytic-phosphate binding site.[13] The site B of PTP1B differs from that of TCPTP by a few amino acids (F52Y and A27S) and thus offers an opportunity to improve selectivity over TCPTP.[16] Based upon this dual binding site concept, recently, we reported, effect of bidentate *p*Tyr mimetics (Difluoromethylphosphonates (DFMP)/Difluoromethylsulfonamide (DFMS)/Isothiazolidinone (IZD)) on benzotriazole-scaffold which lead to a discovery of an orally active dual binding-site PTP1B inhibitors (Compound **1**; Fig. 1).[17] As a part of our ongoing research on PTP1B inhibitors, herein, we report incorporation of monodentate *p*Tyr mimetics (DFMP/DFMS/IZD) on triaryl sulfonamide based-scaffold (Compound **2**; Fig. 1).[18] Although selectivity, pharmacodynamic (PD) and pharmacokinetic (PK) profile of compound **2** is not reported, however, in vitro it showed good PTP1B inhibitory activity (IC_{50}: 74 nM).[18] Therefore, the triaryl sulfonamide based scaffolds were specifically selected to design dual binding PTP1B inhibitors (**5a**–**r**). The in vitro PTP1B inhibitory activity and subtypes-selectiv

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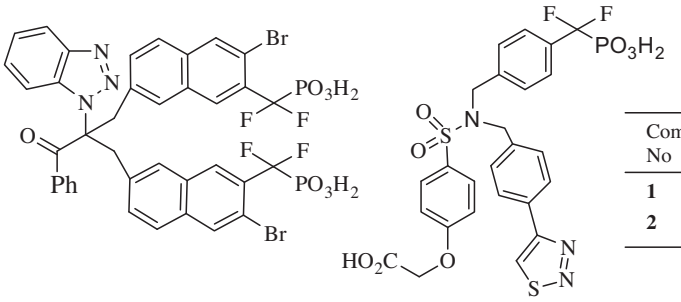


Compd No	PTP1B (IC_{50}; μM)	TCPTP (IC_{50}; μM)	Selectivity
1	0.005	0.580	116
2	0.074	NA	NA

1 **2**

Figure 1. Structurally diverse small molecule based-PTP1B inhibitors.

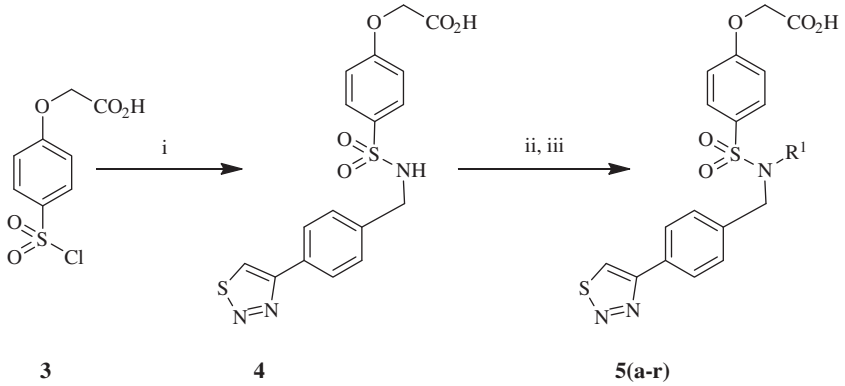
ity of all the test compounds **5a**–**r** were assessed using enzymatic assay (in vitro).[19] Furthermore, based on the in vitro results, highly potent and selective test compounds **5o** and **5p** were subjected in vivo, to determine their antidiabetic effect and PK profile.[20,21]

Synthesis of the title compounds **5a**–**r** was carried out as depicted in Scheme 1, following the modified literature procedure.[18c] Treatment of substituted benzenesulfonyl chloride **3** with primary amine in the presence of *N*,*N*-diisopropylethylamine gave mono-substituted sulfonamide **4**. Alkylation of the compound **4** with appropriate electrophile gave the di-substituted sulfonamides, which were subsequently converted to **5a**–**r** by treatment with trimethylsilyl bromide/trifluoracetic acid to hydrolyze the *t*-butyl esters and the phosphonate diethylesters. All the titled compounds and intermediates were characterized with their physical, analytical and spectral data (^{13}C NMR, ^{1}H NMR and ESI MS). Elemental analyses were determined within 0.04% of theoretical values. Overall, **5a**–**r** was prepared in good yield, under the mild reaction conditions. The percentage yield in the final step was found to be in the range of 60–80%. The ESI MS and NMR spectral data of all the synthesized compounds were also found to be in conformity with the structures assigned and ensure the formation of the compounds **5a**–**r** (see Supplementary data for analytical and spectral data).

The in vitro PTP1B inhibitory activity (*p*-NPP assay) was determined in order to establish the structure–activity relationship (SAR). As shown in Tables 1 and 2, two series of the compounds were prepared by substituting DFMP/DFMS/IZD-benzyl groups (**5a**–**j**) or with DFMP/DFMS-substituted naphthyl/quinolinyl templates (**5k**–**r**) at position R^1. In the first series (**5a**–**j**), two sets of compounds were prepared by substituting DFMP/DFMS benzyl groups (**5a**–**f**) or IZD-substituted benzyl templates (**5g**–**j**), while in the second series (**5k**–**r**), two sets of compounds were prepared by substituting DFMP/DFMS-substituted naphthyl/quinolinyl templates (**5k**–**n**) or its fluoro analogues (**5o**–**r**). Depending on the nature of the substitutents, the tested compounds showed varying degrees of PTP1B inhibitory activity (Tables 1 and 2).

Within the first series (**5a**–**j**), the first set of compounds (**5a**–**f**) containing DFMP-substituted benzyl groups at R^1 (**5a**–**d**) showed diverse PTP1B inhibitory activity depending on the DFMP group position (*meta* vs *para*) on the benzyl ring and the *ortho* substitutents (hydrogen or halogen). Compound **5b**, with a *m*-DFMP group, showed weak PTP1B inhibitory activity relative to that of *p*-DFMP (**2**), whereas compound **5a**, containing *p*-DFMP and *o*-bromo substitution to DFMP, showed good PTP1B inhibitory activity. Replacement of *o*-bromo analogues (**5a**–**b**) with more electronegative *o*-fluoro derivatives (**5c**–**d**), showed improved PTP1B inhibitory activity. Bioisosteric replacement of the *p*-DFMP group in compound **5a** with *p*-DFMS (**5e** and **5f**) showed comparable in vitro PTP1B inhibitory activity, suggesting that the highly negatively charged DFMP group can be replaced with a DFMS group to overcome the issue of low permeability. Among the **5e** and **5f** tested, **5f** showed improved PTP1B inhibitory activity, which could be due to increase in the electronegativity and decrease in the steric bulk (**5e**: ortho-bromo vs **5f**: ortho-fluoro) adjacent to the DFMS group. The second set of compounds containing IZD-substituted benzyl groups at R^1 (**5g**–**j**) showed weak PTP1B inhibitory activity, irrespective of their ortho substitutents. Among the second set (**5g**–**j**), ortho-fluoro derivative (**5j**) was found to be most potent, whereas the ortho-methyl (**5h**) and ortho-bromo (**5i**) derivatives were found to be equipotent. Unsubstituted derivative (**5g**) was found to be least active, with the set.

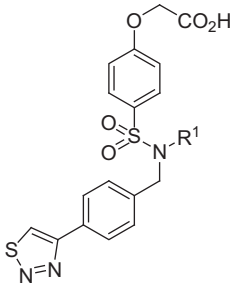
The second series of compounds with position R^1 as DFMP/DFMS-substituted naphthyl/quinolinyl templates (**5k**–**r**) showed potent PTP1B inhibitory activity. Within the second series, the first set of compounds containing DFMP-substituted naphthyl/quinoli-

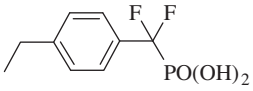
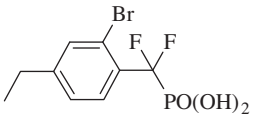
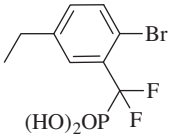
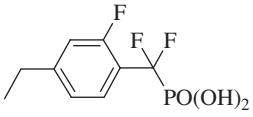
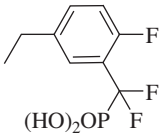
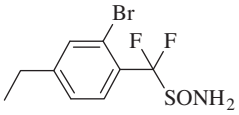
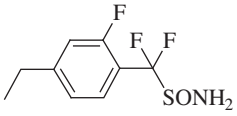
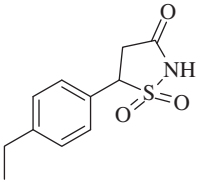
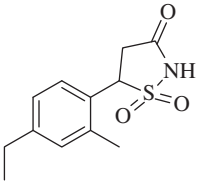
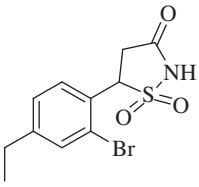


3 **4** **5(a-r)**

Scheme 1. Synthesis of compounds **5a**–**r**. Reagents and conditions: (i) thiadiazolyl-C_6H_4-CH_2-NH_2, CH_2Cl_2, DIEA, 25 °C, 5 h; (ii) R^1-X, K_2CO_3, CH_3CN, 70 °C, 4 h; (iii) TMSBr, CH_2Cl_2, −15 °C, 3 h or TFA, CH_2Cl_2, 2 h.

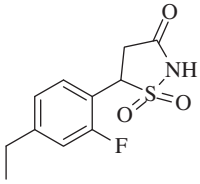
Table 1
The In vitro PTP1B inhibitory activity and selectivity of test **5a–j**



Compd No.	R^1	PTP1B[a] IC_{50} (nM)	TCPTP[b] IC_{50} (nM)
2		78 ± 02	546 ± 12 (~7)[c]
5a		50 ± 03	750 ± 13 (~15)[c]
5b		288 ± 08	
5c		35 ± 04	870 ± 07 (~24)[c]
5d		141 ± 10	
5e		47 ± 04	
5f		31 ± 02	620 ± 08 (~20)[c]
5g		500 ± 10	
5h		470 ± 11	
5i		480 ± 11	

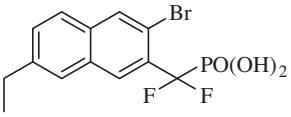
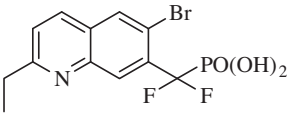
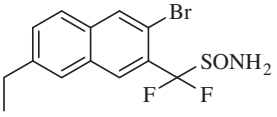
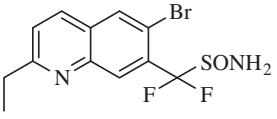
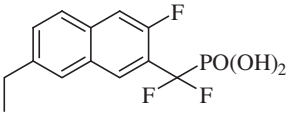
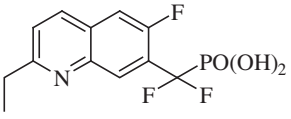
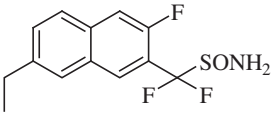
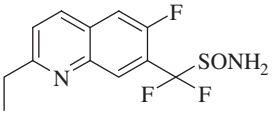
(*continued on next page*)

Table 1 (*continued*)

Compd No.	R^1	PTP1B[a] IC_{50} (nM)	TCPTP[b] IC_{50} (nM)
5j		460 ± 12	

[a] Enzymatic assay was carried out in 96-well plates. The initial rate of PTPase-catalyzed hydrolysis of pNPP was measured at 405 nm. IC_{50} value was determined under fixed pNPP concentration of 1 mM (n = 3; represents Mean ± SD).
[b] Selected test compounds which were screened for TCPTP inhibitory activity also showed >5000-fold selectivity over CD45, LAR, SHP-1 and SHP-2 enzymes (data not shown).
[c] Fold selectivity calculated as ratio of average IC_{50} values of TCPTP/PTP1B inhibitions.

Table 2
The In vitro PTP1B inhibitory activity and selectivity of test **5k–r**

Compd No.	R^1	PTP1B[a] IC_{50} nM	TCPTP[b] IC_{50} nM
5k		17 ± 01	
5l		18 ± 03	
5m		39 ± 03	
5n		41 ± 05	
5o		9 ± 01	870 ± 05 (∼96)[c]
5p		11 ± 04	990 ± 08 (∼90)[c]
5q		35 ± 07	
5r		38 ± 08	

[a] Enzymatic assay was carried out in 96-well plates. The initial rate of PTPase-catalyzed hydrolysis of pNPP was measured at 405 nm. IC_{50} value was determined under fixed pNPP concentration of 1 mM (*n* = 3; represents Mean ± SD).
[b] Selected test compounds which were screened for TCPTP inhibitory activity also showed >5000-fold selectivity over CD45, LAR, SHP-1 and SHP-2 enzymes (data not shown).
[c] Fold selectivity calculated as ratio of average IC_{50} values of TCPTP/PTP1B inhibitions.

nyl templates (**5k–l)** showed better PTP1B inhibitory activity compare to its bioisostere DFMS-substituted templates (**5m–n**). At pH 6.5 (simulate with in vitro system), it has been reported that the monoanionic DFMP exhibit an equilibrium with its dianionic form and the PTP1B enzyme show strong binding interaction with the dianionic form.[22] Thus compounds containing DFMP-substituted templates (**5k–l)** showed better PTP1B inhibitory activity compare to DFMS-substituted templates (**5m–n**), may be due to the preference of PTP1B enzyme binding with the dianionic form of DFMP. In second set, replacement of bromo analogues (**5k–n**) with its more electronegative fluoro analogues (**5o–r**), leads to a highly potent PTP1B inhibitors among all series. As observed in the previous series, electronegative fluoro analogues (**5o–r**) were found to be more potent compare to its bromo analogues (**5k–n**), which could

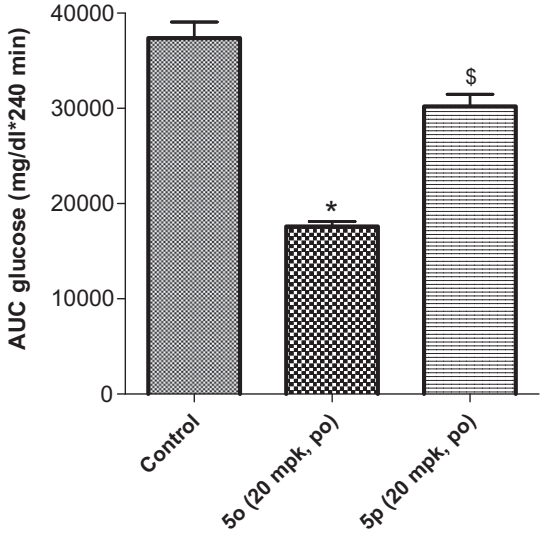
be due to increase in the electronegativity and decrease in the steric bulk (**5k**–**n**: ortho-bromo vs **5o**–**r**: ortho-fluoro) adjacent to the DFMS/DFMP groups.

The SAR study reveals that the *para*-substituted *p*Tyr mimetics exhibit favorable PTP1B inhibitory activity. The compounds with *ortho*-halogen substitution next to *p*Tyr mimetics showed potent PTP1B inhibitory activity and among three-different *p*Tyr mimetics, DFMP exhibited the highest PTP1B inhibitory activity. Similarly, the fluoro analogues of naphthyl/quinolinyl templates containing DFMP as a *p*Tyr mimic were found to be more potent than their corresponding bromo analogues. Among two series tested, compounds with DFMP/DFMS-substituted naphthyl/quinolinyl templates (**5k**–**r**; second series) were found to be more potent than the benzyl derivatives (**5a**–**j**; first series). One possible explanation for this is that naphthyl/quinolinyl ring possibly involves more pi-stacking interactions with aromatic residues located close to the active site of PTP1B, compare to benzyl ring. Overall, in vitro PTP1B inhibitory activity results clearly suggest that the potency of triaryl sulfonamide-based PTP1B inhibitors can be modulated using suitable substitutents at R^1 position.

The in vitro selectivity over PTPs (PTPα, LAR, CD45, VHR, SHP-1, SHP-2 and TCPTP) was evaluated for most potent compounds (**5a**, **5c**, **5f**, **5o** and **5p**), using *p*-NPP assay and IC_{50} values are listed in (Tables 1 and 2).[19] Compounds **5a** and **5c** showed ~15 to 25-fold selectivity, **5f** showed ~20-fold selectivity, **5o** and **5p** showed ~90 to 96-fold selectivity over TCPTP enzyme, while all the selected test compounds showed >5,000-fold selectivity over PTPα, LAR, CD45, VHR, SHP-1 and SHP-2 enzymes. The compounds **5a**, **5c** and **5f** containing R^1 as DFMP/DFMS-substituted benzyl groups showed poor selectivity. Compounds **5o** and **5p** containing DFMP/DFMS-substituted naphthyl/quinolinyl templates at R^1, showed excellent selectivity over TCPTP, indicated that among the two different ring systems (benzyl/fused-ring system) selected as R^1, only fused-ring templates (naphthyl/quinolinyl derivatives) showed best selectivity. The in vitro PTP1B inhibitory activity and PTPs selectivity was also determined for standard compound **2** (Table 1; IC_{50}: 78 ± 02 nM, with ~7-fold selectivity over TCPTP) and the IC_{50} values (PTP 1B inhibitory activity) were found to be in agreement with the literature (IC_{50}: 74 nM).[18]

The in vivo antidiabetic activity of the most potent and selective compounds (**5o** and **5p**; @ 20 mpk, po) was evaluated in male C57BL/6J mice, using IPGTT (Intraperitoneal glucose tolerance test) protocol and changes in serum glucose levels (AUC glucose @ 240 min; mg dL^{-1}), with compounds **5o** and **5p** are shown in Figure 2.[20] Compound **5o** showed excellent antidiabetic activity orally, whereas compound **5p** showed moderate activity upon oral administration (−52.8 ± 5.36 and −19.18 ± 5.8, respectively). In vivo antidiabetic activity was also evaluated for standard compound **2** (as positive control). Compared to vehicle control, there was no change in the serum glucose levels (AUC glucose) was observed with compound **2**, when it was administered @ 20 mpk, po. Compound **2** was found to be inactive, which could be due to its poor oral bioavailability.

Further to understand the pharmacokinetic (PK) profile, a comparative single dose (20 $mgkg^{-1}$ iv or po) study of **5o** and **5p** was carried out in male C57BL/6J mice ($n = 6$) and the various PK parameters such as T_{max}, $t_{1/2}$, C_{max}, AUC and %*F* were calculated and recorded (Table 3).[21] In a single-dose PK study, compound **5o** showed rapid t_{max}, good area under the curve (AUC), and extended half-life ($t_{1/2}$), whereas **5p** showed extended t_{max}, short $t_{1/2}$ and moderate AUC. Relative to **5p**, compound **5o** showed ~7-fold



*P<0.05, $P<0.01, Two-Way ANOVA followed by Bonferroni post test, M ± SEM;
Standard compound **2** was also tested @ 20 mpk, po, but no change in the AUC glucose was observed with respect to vehicle control.

Figure 2. In vivo antidiabetic activity of compounds **5o** and **5p** in C57 mice (IPGTT).

Table 3
Pharmacokinetic (PK) study parameters[a] of compounds **5o** and **5p**

Compd No.	T_{max} (h)	C_{max} (μg/ml)	$T_{1/2}$ (h)	AUC (0–∞) h μg/ml	%*F*[b]
5o	0.31 ± 0.01	6.3 ± 0.51	6.96 ± 0.91	8.91 ± 0.14	6.51
5p	0.69 ± 0.10	0.96 ± 0.32	0.99 ± 0.76	1.32 ± 0.31	0.9

[a] In male C57BL/6 J mice ($n = 6$), compounds **5o** and **5p** were administered orally (po) at 20 $mgkg^{-1}$ dose and plasma concentration was analyzed by LC–MS, values indicate Mean ± SD.
[b] Oral bioavailability (%F) was calculated wrt to iv AUC (**5o**: 137.07 ± 4.28 and **5p**: 146.66 ± 9.81 h μg/ml) administered at 20 $mgkg^{-1}$ dose, iv.

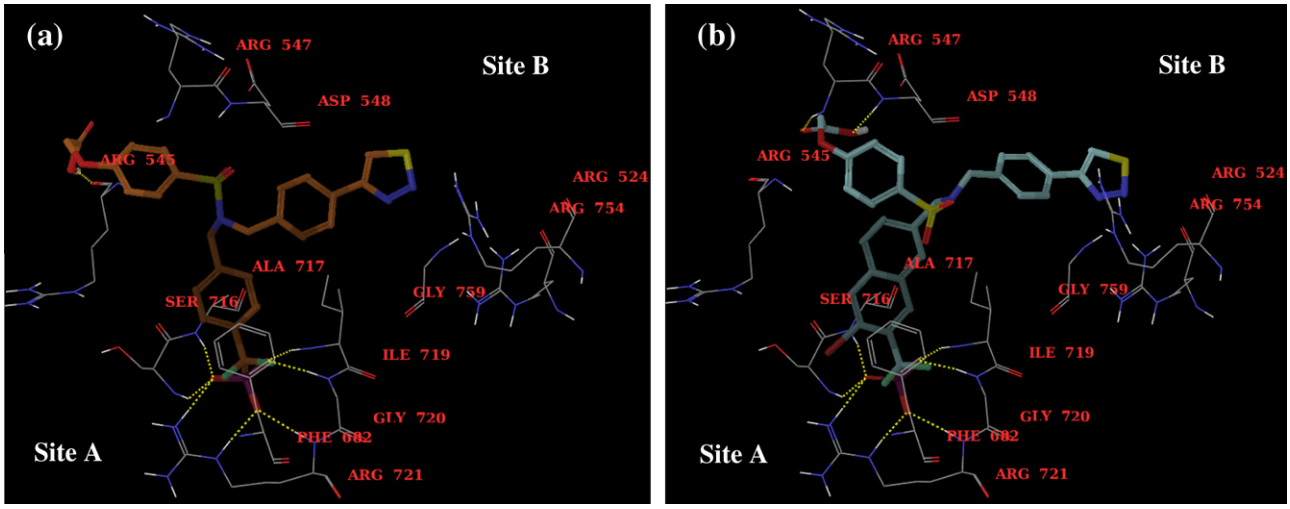


Figure 3. Key interaction of compounds **2** and **5o** with active site-A and secondary aryl-binding site-B. (a) Binding pose of compound **2** in the PTP1B active site is indicated, wherein it interact closely with key residues of site A and marginally with site B. (b) Compound **5o** docks very well into both the sites (A & B), particularly, flipping of thiadiazolyl moiety in site B and its strong interactions with key resides of site B (Asp548, Arg524 and Arg754) favors best fits of **5o**. Residue numbering is as per current numbering (PDB code: 1Q6T): Arg24 (524), Asp48 (548) and Arg254 (754).

higher bioavailability (%*F*: ~6.5%). Thus improved pharmacokinetic profile of compound **5o** justifies its excellent pharmacodynamic effects (antidiabetic activity) in C57 mice, when administered orally.

The molecular docking analysis of **5o** was carried in Glide, to understand its critical interactions with both the binding sites (A and B) of PTP1B enzyme.[23] The initial Glide docking studies for **5o** gave poor results in terms of binding conformation. Based on this observation, the compound **5o** was docked using the induced fit docking (IFD) protocol. The IFD is based on the docking program Glide with the refinement module in Prime (Schrodinger, Inc.), which was reported to accurately predict the ligand binding modes and concomitant structural changes in the receptor.[23]

Since the interaction of compound **2** with the PTP1B enzyme is unpublished, molecular docking analysis was also carried out for compound **2** (Fig. 3a). As described earlier, for achieving selectivity over TCPTP, interactions in site B is essential. The IFD results illustrate that compound **2** completely docks in the binding site A and the DFMP substituted phenyl ring of compound **2** strongly coordinate with Phe682 (site A residue), while the oxy acetic acid and thiadiazolyl ring of compound **2** partially project in site B and interact partly with key residues of site B. In vitro, ~7-fold selectivity over TCPTP enzyme was observed with compound **2** and it could be attributed to its partial orientation in site B, which is essential for achieving selectivity over TCPTP enzyme.

The IFD results of compound **5o** illustrated that introduction of DFMP-naphthyl template allows to adopt new conformation; as a result, it docks very well at the both binding sites (Fig. 3b). It was observed that upon IFD, flipping of thiadiazolyl ring was observed in site B and because of this change, compound **5o** docks very well into both the sites (A and B). Thus the PTP1B selectivity over TCPTP was achieved by taking advantage of amino acid differences in the site B. In particular, the flipping of thiadiazolyl moiety in site B and its strong interaction with key resides of site B (Asp548, Arg524 and Arg754). The favorable hydrogen bond interactions of compound **5o** with both the sites (A and B) of PTP1B enzyme support its potent in vitro PTP1B activity and excellent selectivity over TCPTP.

In summary, novel triaryl sulfonamide-based PTP1B inhibitors containing DFMP-substituted naphthyl template at R^1 show excellent in vitro potency and selectivity over TCPTP, indicating that among three different ring systems selected as R^1, only naphthyl derivatives shows best selectivity, due to favorable orientation of ligand across both the binding sites of the PTP1B enzyme. The lead compound **5o** shows significant antihyperglycemic effects (in vivo), along with oral bioavailability. Thus, preliminary study results confirm that highly potent and selective PTP1B inhibitor could be viable approach for the effective treatment of T2DM.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.122.

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21. Briefly, for single dose PK study, test compounds were administered orally/iv on a body weight basis (20 $mgkg^{-1}$) to overnight fasted male C57BL/6J mice. Serial blood samples were collected in microcentrifuge tubes containing EDTA at pre-dose, 0.15, 0.3, 0.5, 0.75, 1, 2, 4, 6, 8, 24 and 30 h post-dose after compounds administration. Approximately 0.3 ml of blood was collected at each time point and centrifuged at 4 °C. The obtained plasma was frozen, stored at −70 °C and the concentrations of compounds in plasma were determined by the LC–MS/MS (Shimadzu LC10AD, USA), using YMC hydrosphere C18 (2.0×50 mm, 3 μm) column (YMC Inc., USA). The pharmacokinetic parameters, such as T_{max}, $t_{1/2}$, C_{max}, AUC and *%F* were calculated using a non-compartmental model of WinNonlin software version 5.2.
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