DOI: 10.1002/cmdc.201100077

Discovery of Orally Active, Potent, and Selective Benzotriazole-Based PTP1B Inhibitors**

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The worldwide incidence of metabolic syndromes such as obesity and diabetes are increasing at an alarming rate.[1,2] Patients that suffer from obesity-induced type 2 diabetes (informally known as *diabesity*) are at increased risk of cardiovascular disease; their numbers pose a significant economic burden on health services.[3] Type 2 diabetes mellitus (T2DM) is clinically characterized by increased blood glucose levels, either due to defects in insulin secretion, insulin resistance, or both.[4] Current treatments for diabetic patients include various oral antihyperglycemic agents; however, over a period of time nearly half of T2DM sufferers lose their response to these agents and thereby require insulin therapy. Except incretin therapies, most of the available anti-hyperglycemic 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 that can complement existing therapies and prevent the progression of secondary complications associated with diabesity.

In recent years, development of protein tyrosine phosphatase 1B (PTP1B) inhibitors has been considered as one of the best validated biological targets for the treatment of T2DM.[10] PTP1B acts as a negative regulator in insulin signaling pathways; it dephosphorylates key tyrosine residues within the regulatory domain of the β-subunit of the insulin receptor.[11] Thus, the inhibition of PTP1B activity has the potential for enhancing insulin action by prolonging the phosphorylated state of the insulin receptor.[12] Gene knockout studies in animals have also demonstrated that PTP1B$^{-/-}$ mice show increased insulin sensitivity and are resistant to diet-induced obesity.[13,14]

Over the past two decades, several structurally diverse small-molecule-based PTP1B inhibitors have been developed, including Ertiprotafib, which was discontinued in phase II clinical trials owing to lack of efficacy and dose-dependent side effects.[15] Most of the initial PTP1B inhibitors, such as phosphonates, carboxylic acids, and difluoromethylphosphonates (DFMPs), were designed to bind to the active site (site 1/A) by mimicking the phosphotyrosine (pTyr) substrate.[16] 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 challenges, as most of the closely associated PTPs, particularly TCPTP, share a high degree of primary sequence identity (92%) in the active site (pTyr binding pocket).[17] Lack of oral bioavailability is another important issue in the development of potent and selective PTP1B inhibitors, as the majority of the active-site-directed PTP1B inhibitors exhibit limited cell permeability due to the presence of negatively charged polar groups.[18,19]

To address this problem, Zhang and colleagues identified an additional noncatalytic aryl phosphate binding site (site 2/B) proximal to the catalytic phosphate binding site.[17] 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.[20] Consequently, dual-site inhibitors were designed to bind across both sites A and B, to achieve additive effects and thereby improve potency and selectivity toward PTP1B over closely associated PTPs.[21] Based on this dual binding site concept, various DFMP-based PTP1B inhibitors such as arylketone **1**, benzotriazoles **2a** and **2b**, and naphthyl derivative **3** were developed (Figure 1).[22] The X-ray crystal structure of PTP1B in complex with compound **2b** reveals that sites A and B each have a DFMP moiety anchored into it.[23] The benzotriazole ring system also functions as an anchor and is located under the YRD loop, thereby rigidly locking the molecule into the active site and providing good selectivity for PTP1B over other PTPs. The fourth substituent (benzene ring) occupies a hydrophobic pocket. Altogether, this signifies that the presence of all four substituents oriented rigidly by the molecule's stereocenter is essential for high potency and selectivity.[22]

Although results of oral bioavailability and in vivo antidiabetic activity assays for compound **2a** have yet to be published, in vitro results show improved PTP1B inhibitory activity (IC_{50} = 5 nM) and moderate selectivity (sevenfold) over TCPTP (IC_{50} = 36 nM). The X-ray crystal structure of PTP1B in complex with compound **2a** illustrates that a methoxy group aligns very closely (3.7 Å) to the side chain of F52 (site B).[23] Oral administration of compounds **1** and **3** demonstrated good antidiabetic activity (compound **3**: ED_{50} = 0.8 mg kg^{-1}, p.o.) and oral bioavailability (compounds **1** and **3:** F = 13 and 24%, respectively) in different animal species, despite their moderate in vitro PTP1B inhibitory activity (IC_{50} = 120 nM) and poor selectivity

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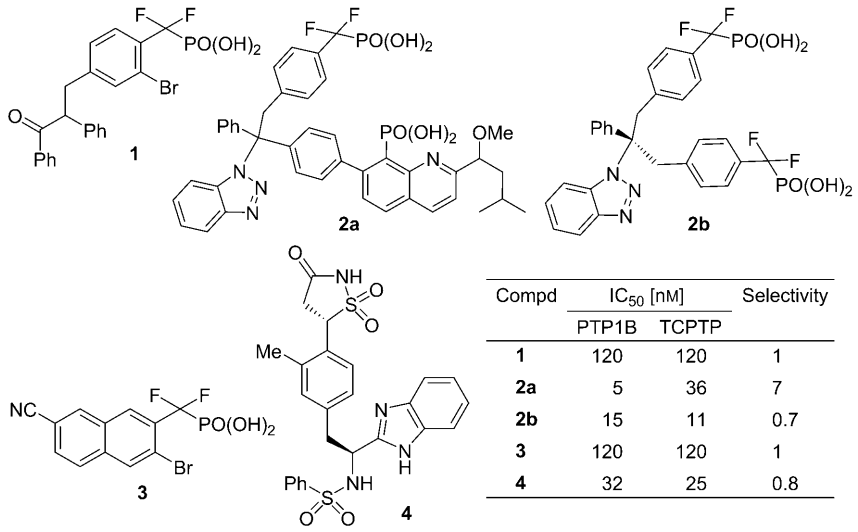
[**] ZRC communication No. 378 (part of PhD thesis work of D.P.)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201100077.



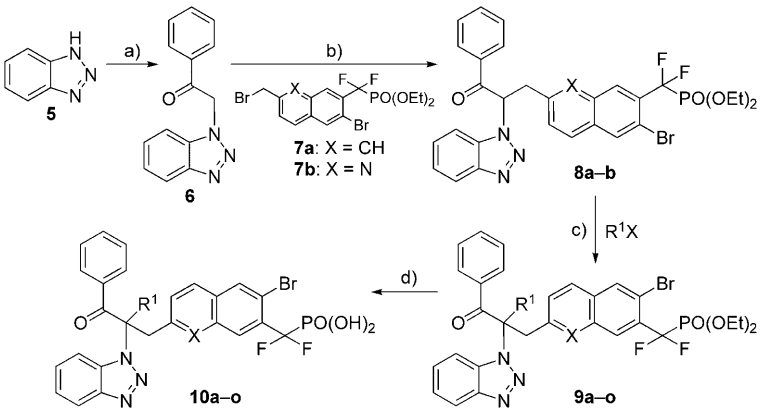
Compd	IC_{50} [nM]		Selectivity
	PTP1B	TCPTP	
1	120	120	1
2a	5	36	7
2b	15	11	0.7
3	120	120	1
4	32	25	0.8

Figure 1. Structurally diverse small-molecule-based PTP1B inhibitors **1**–**4**.

(< 1-fold over TCPTP).[22] A few years ago researchers at Incyte Ltd. (Wilmington, DE, USA) reported isothiazolidinone (IZD)-based pTyr mimetics as potent PTP1B inhibitors (Figure 1, **4**: $IC_{50} = 32$ nM).[24] The IZD heterocycles were designed based on the hypothesis that the two sulfonyl oxygen atoms mimic the oxygen atoms of the DFMP group, whereas the carbonyl and the ionized NH groups mimic the DFMP anion.[25] Most importantly, compounds bearing this diffusely mono-anionic heterocyclic pTyr mimetic (IZD) showed improved membrane permeability and high binding affinity toward site B of PTP1B.[26]

With the development of potent and selective PTP1B inhibitors as a potentially viable approach for the safe and effective treatment of T2DM, herein we report the design of novel benzotriazole-based dual-site PTP1B inhibitors (compound **10a**–**o**). There are four key structural components: a) benzotriazole ring, b) acetophenone, c) DFMP-substituted naphthyl or quinolinyl derivatives, and d) benzyl, naphthyl, or quinolinyl ring systems suitably substituted with difluoromethylsulfonamide (DFMS), DFMP, or IZD groups. The benzotriazole ring system was introduced as a basic pharmacophore to obtain superior PTP1B selectivity over other PTPs.[22] The two DFMP groups were specifically incorporated as pTyr mimetics to access both binding sites A and B, thereby improving potency and selectivity for PTP1B over TCPTP. Furthermore, bioisosteric replacement of DFMP was carried out with DFMS/IZD, to improve membrane permeability and binding affinity for site B.[26] The acetophenone, benzyl, naphthyl, and quinolinyl ring systems were integrated to improve overall lipophilicity and thus oral efficacy. The in vitro PTP1B inhibitory activity and subtype-selectivity of all test compounds **10a**–**o** were assessed with an in vitro *para*-nitrophenylphosphate (*p*NPP) enzyme assay.[27] Furthermore, based on the in vitro results, highly potent and selective test compounds **10h** and **10j** were subjected to in vivo studies to determine their antidiabetic effects and pharmacokinetic (PK) profiles.[28]

Synthesis of the title compounds **10a**–**o** was carried out as shown in Scheme 1 by following a modified published procedure.[22b] Treatment of benzotriazole **5** with phenacyl bromide in the presence of sodium hydride gave the 1-phenacyl-1*H*-1,2,3-benzotriazole **6**. Deprotonation of compound **6** with *n*-butyllithium followed by alkylation with the appropriate electrophiles (**7a** or **7b**) gave the intermediate **8a**,**b** in good yield. Subsequent alkylation of compounds **8a**,**b** with variously substituted benzyl bromides or the naphthyl or quinolinyl analogues in the presence of *n*-butyllithium led to the formation of compounds **9a**–**o**, which, upon deprotection with trimethylsilyl bromide/trifluoroacetic acid, yielded the final compounds **10a**–**o**. The structures of all final compounds and their intermediates were confirmed by physical, analytical, and spectral data (^{13}C NMR, ^{1}H NMR, and ESIMS). In general, compounds **10a**–**o** were prepared in good yields under mild reaction conditions. The overall yields at the final step were found to be in the range of 60–80%. ESIMS data showed molecular ion peaks $[M]^+$ with different intensities, corresponding to the molecular weights of the compounds. Elemental analyses were determined within ±0.04% of theoretical values. The IR and NMR spectroscopic data for all synthesized compounds were also found to be in agreement with the structures assigned (see the Supporting Information).



Scheme 1. *Reagents and conditions:* a) $BrCH_2COPh$, NaH, DMF, 25 °C, 5 h; b) *n*BuLi, THF, −78–25 °C, 4 h; c) *n*BuLi, THF, R^1X −78–25 °C, 6 h; d) TMSBr, CH_2Cl_2, −15 °C, 3 h and/or TFA, CH_2Cl_2, 2 h.

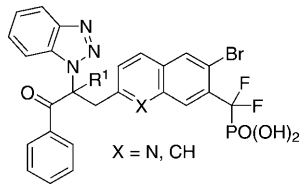
The in vitro PTP1B inhibitory activity (*p*NPP assay) was determined in order to establish the structure–activity relationship

(SAR). The compounds were prepared in two main series, either by introducing naphthyl (**10a**–**k**) or quinolinyl (**10l**–**o**) templates. Within the first series **10a**–**k**, distinct sets of compounds were prepared by substituting DFMP/DFMS/IZD benzyl groups at position R^1 (**10a**–**g**) or with DFMP/DFMS-substituted naphthyl/quinolinyl templates (**10h**–**k**). In the second series **10l**–**o**, DFMP/DFMS-substituted quinolinyl analogues of the selected test compounds of the first series (**10b**, **10d**, **10i**, and **10k**) were prepared. As shown in Table 1, all the tested compounds showed varying degrees of PTP1B inhibition (IC_{50}) depending on the nature of the substituents.

The first set of compounds containing DFMP-substituted benzyl groups at R^1 (compounds **10a**–**c**) showed diverse PTP1B inhibitory activity depending on the DFMP group position (*meta* versus *para*) on the benzyl ring and the *ortho* substituents (hydrogen or halogen). Compound **10c**, with a *m*-DFMP group, showed weak PTP1B inhibitory activity relative to that of *p*-DFMP (in **10a**), whereas compound **10b**, containing *p*-DFMP and *o*-bromo substitution to DFMP, showed good PTP1B inhibitory activity. Bioisosteric replacement of the *p*-DFMP group in compound **10b** with *p*-DFMS (**10d**) showed similar in vitro PTP1B inhibitory activity, indicating that the highly negatively charged DFMP group can be replaced with a DFMS group to overcome the issue of low permeability.

The second set of compounds containing IZD-substituted benzyl groups at R^1 (**10e**–**g**) showed weak PTP1B inhibitory activity, irrespective of their *ortho* substituents. The third set of the compounds with position R^1 as DFMP/DFMS-substituted naphthyl/quinolinyl templates (**10h**–**k**) showed potent PTP1B inhibitory activity. The second series of compounds (DFMP/DFMS-substituted quinolinyl derivatives **10l**–**o**) showed promising and similar PTP1B inhibitory activities as observed for the

Table 1. In vitro PTP1B inhibitory activity and selectivity of test compounds **10a**–**o**.



IC_{50} [nM]

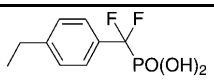
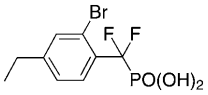
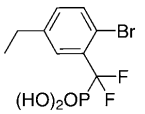
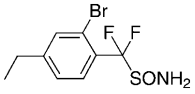
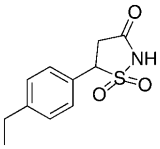
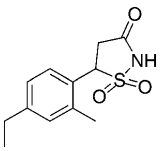
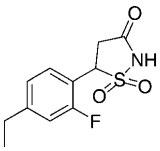
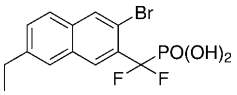
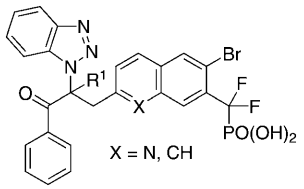
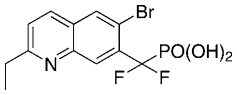
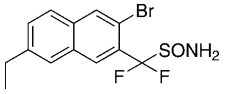
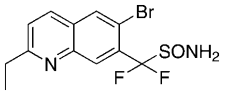
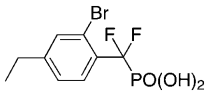
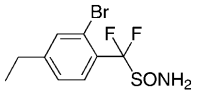
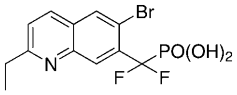
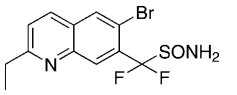
Compd	R^1	X	PTP1B[a]	TCPTP[b]
10a		CH	46	
10b		CH	20	201 (10)[c]
10c		CH	404	
10d		CH	19	192 (10)[c]
10e		CH	930	
10f		CH	924	
10g		CH	919	
10h		CH	5	580 (116)[c]

Table 1. (Continued)



IC_{50} [nM]

Compd	R^1	X	PTP1B[a]	TCPTP[b]
10i		CH	3	90 (30)[c]
10j		CH	4	480 (120)[c]
10k		CH	6	185 (30.8)[c]
10l		N	21	
10m		N	22	
10n		N	5	101 (20.2)[c]
10o		N	4	82 (20.5)[c]

[a] Enzyme assays were carried out in 96-well plates; *para*-nitrophenylphosphate (*p*NPP) and test compounds were added to assay buffer, and reactions were initiated by the addition of PTP1B or TCPTP (10–100 nM). The initial rate of PTPase-catalyzed hydrolysis of *p*NPP was measured at λ 405 nm. IC_{50} values were determined under a fixed *p*NPP concentration of 1 mM ($n=3$). [b] Selected test compounds that 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 the ratio of IC_{50} values of TCPTP/PTP1B inhibition.

DFMP/DFMS-substituted naphthyl derivatives **10b**, **10d**, **10i**, and **10k**.

As mentioned above, our goal was to develop potent and selective tetrasubstituted benzotriazole-based PTP1B inhibitors, and in this attempt in the first series, three sets of compounds were prepared by introducing DFMP, DFMS, or IZD as pTyr mimetics either on benzyl or naphthyl/quinolinyl templates. The SAR study revealed that the *para*-substituted pTyr mimetics (DFMP, DFMS, and IZD) exhibit favorable PTP1B inhibitory activity. The compounds with *ortho*-bromo substitution next to pTyr mimetics show potent PTP1B inhibitory activity, and among three different pTyr mimetics, DFMP and DFMS exhibited the highest PTP1B inhibitory activity. No significant difference in activity was observed among the compounds with naphthyl or quinolinyl templates. However, these compounds were found to be more potent than the benzyl derivatives. Overall, the in vitro PTP1B inhibition results clearly show that the potency of tetrasubstituted dual-site benzotriazole-based PTP1B inhibitors can be modulated with suitable substituents at the R^1 position.

The in vitro selectivity over other PTPs (PTPα, LAR, CD45, VHR, SHP-1, SHP-2, and TCPTP) was evaluated for the most potent compounds (**10b**, **10d**, **10h**–**k**, **10n**–**o**) by using the *p*NPP assay, and IC_{50} values are listed in Table 1.[27] Compounds **10b** and **10d** showed ~10-fold selectivity, **10h** and **10j** showed >115-fold selectivity, **10i** and **10k** showed ~30-fold selectivity, and compounds **10n**–**o** showed ~20-fold selectivity over TCPTP. All the selected test compounds showed >5000-fold selectivity over PTPα, LAR, CD45, VHR, SHP-1, and SHP-2 enzymes. Compounds **10b** and **10d**, containing DFMP/DFMS-substituted benzyl groups at R^1, showed poor selectivity. Compounds **10i** and **10k**, containing DFMP/DFMS-substituted quinolinyl templates at R^1, and the second series of compounds **10n**–**o**, containing additional DFMP/DFMS-substituted quinolinyl templates, showed moderate TCPTP selectivity, while **10h** and **10j**, containing DFMP/DFMS-substituted naphthyl templates at R^1, showed excellent selectivity over TCPTP, indicating that among the three different ring systems (benzyl, naphthyl, and quinolinyl) selected as R^1, only naphthyl derivatives showed the best selectivity, perhaps due to the favorable orientation of the naphthyl ring system across both the binding sites A and B of PTP1B.

The in vivo antidiabetic activity of the most potent and selective compounds **10h** and **10j** was evaluated in male C57BL/6J mice using the intraperitoneal glucose tolerance test (IPGTT) protocol.[28] Briefly, mice fasted overnight ($n=6$) were dosed orally (p.o.)/intraperitoneally (i.p.) with the test compounds **10h** or **10j** (10 mg kg^{-1}) 0.5 h prior to the i.p. glucose load (1.5 g kg^{-1}, 10 mL). Blood samples were collected at various time points (0, 30, 60, 120, and 240 min), and the serum was separated, subjected to glucose estimation, and serum glucose levels (mg dL^{-1}) were recorded (Figure 2). Both the test compounds effected significant decreases in blood glucose when administered by the i.p. route. Compound **10h** showed excellent antidiabetic activity orally, whereas compound **10j** showed moderate activity upon p.o. administration. In the design of compound **10j** (R^1: DFMS-substituted naphthyl derivative), it was assumed that the highly negatively charged DFMP group of **10h** could be replaced with a DFMS group to alleviate the permeability issue; however, these comparative oral antidiabetic activity results indicate that the DFMP group is more favorable for oral antidiabetic activity than the DFMS group.

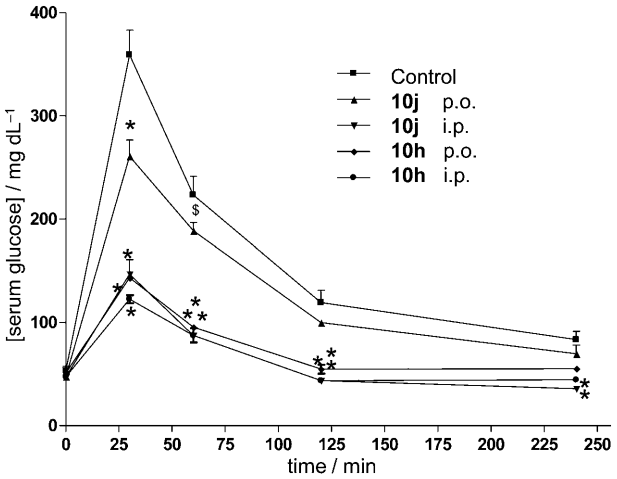


Figure 2. Antidiabetic activity of compounds **10h** and **10j** in C57 mice (IPGTT): Overnight-fasted male C57BL/6J mice ($n=6$) were dosed p.o./i.p. with vehicle or test compounds (10 mg kg^{-1}) 0.5 h prior to IPGTT (1.5 g kg^{-1}, 10 mL); serum glucose levels were determined at 0, 30, 60, 120, and 240 min. Values represent the mean ± SEM; $^{*}p<0.01$ and $^{\$}p<0.05$ by two-way ANOVA followed by Bonferroni post-test.

Further to understand the PK profile, a comparative single dose (10 mg kg^{-1} i.v. or p.o.) study of **10h** and **10j** was carried out in male Wistar rats ($n=6$), and the various PK parameters were recorded (Table 2). In a single-dose PK study, compound **10j** showed rapid t_{max} and clearance, good area under the curve (AUC), and moderate half-life ($t_{1/2}$), whereas **10h** showed extended t_{max} and $t_{1/2}$ values, and moderate AUC and clearance. Relative to **10j**, compound **10h** showed roughly sevenfold higher bioavailability (F ~68%). Thus, the improved PK profile of compound **10h** supports its excellent pharmacodynamic effects (antidiabetic activity) in C57 mice when administered orally.

Table 2. Comparison of pharmacokinetic parameters of compounds **10h** and **10j**.

Route	Parameter[a]	10h	10j
i.v.	$t_{1/2}$ [h]	1.01 ± 0.2	2.41 ± 0.3
	K_{elim} [h^{-1}]	0.99 ± 0.1	0.29 ± 0.1
	AUC [h μg mL^{-1}]	15.499 ± 0.191	29.341 ± 0.795
p.o.	t_{max} [h]	0.33 ± 0.2	1.21 ± 0.01
	$t_{1/2}$ [h]	3.81 ± 0.2	8.67 ± 0.26
	K_{elim} [h^{-1}]	0.44 ± 0.01	0.08 ± 0.05
	AUC [h μg mL^{-1}]	10.562 ± 0.105	3.031 ± 0.312
	F [%]	68.14	10.33

[a] Single-dose (10 mg kg^{-1} i.v./p.o.) PK studies for compounds **10h** and **10j** were carried out in fasted male Wistar rats ($n=6$), and plasma compound concentrations were determined by LC–MS–MS; data represent the mean ± SD.

A molecular docking analysis of **10h** was carried with Glide docking software (ver. 5.6) to understand its selectivity profile and critical interactions with both binding sites A and B of PTP1B.[29] The initial Glide docking studies for **10h** gave poor results in terms of binding conformation. Based on this observation, the compound **10h** was docked in the active site of PTP1B (PDB code: 1Q6T) using the induced-fit docking (IFD) protocol, which involves adjustments in the residues surrounding the binding sites to obtain an alternative structure that can accommodate ligands that would otherwise not fit into the original binding sites. The IFD procedure is based on the Glide docking program with the refinement module in Prime (Schrödinger LLC), which was reported to accurately predict the ligand binding modes and concomitant structural changes in the receptor.[29] Prime was used for side chain rotamer prediction and energy minimization of the conformation of residues located within 5 Å of the ligand in any of the poses.

The IFD results illustrate that the residues of both binding sites A and B adopt new conformations, allowing better ligand fit (**10h**) at both sites. The comparative binding site pockets of PTP1B with respect to compound **10h** and in comparison with the X-ray crystal structure of the known PTP1B inhibitor **2a** are shown in Figure 3A and 3B. It was observed that upon IFD, Arg524 and Phe682 rotamers are changed, and because of this, compound **10h** docks very well into both sites. Thus PTP1B selectivity over TCPTP was achieved by taking advantage of amino acid differences in site B (F552Y and A527S). In particular, the flipping of the Phe682 aromatic ring and the side chain of Arg524 was predominant. As a result, the site B cavity is enlarged, thereby accommodating ligand **10h** quite well, and hence site B best fits with the overall shape of **10h** (Figure 3C). The favorable hydrogen bond interactions between compound **10h** and both sites A and B of PTP1B support its excellent in vitro PTP1B selectivity over TCPTP.

In summary, novel tetrasubstituted benzotriazole-based PTP1B inhibitors containing a DFMP-substituted naphthyl template at R^1 show excellent in vitro potency and selectivity over TCPTP, indicating that among three different ring systems (benzyl, naphthyl, and quinolinyl) selected as R^1, only naphthyl derivatives show high selectivity owing to favorable orientation of the naphthyl ring system across both the binding sites of the PTP1B enzyme. The lead compound **10h** shows excellent anti-hyperglycemic effects in animal models, along with improved oral bioavailability. The results of our in silico docking study are in agreement with the observed in vitro PTP1B selectivity. The results of this preliminary study confirm that highly potent and selective PTP1B inhibitors could represent a viable approach toward the safe and effective regulation of glucose homeostasis in T2DM patients. Further evaluations of these lead compounds (chronic pharmacodynamic studies and toxicological evaluations) are currently underway, and results will be communicated in due course.

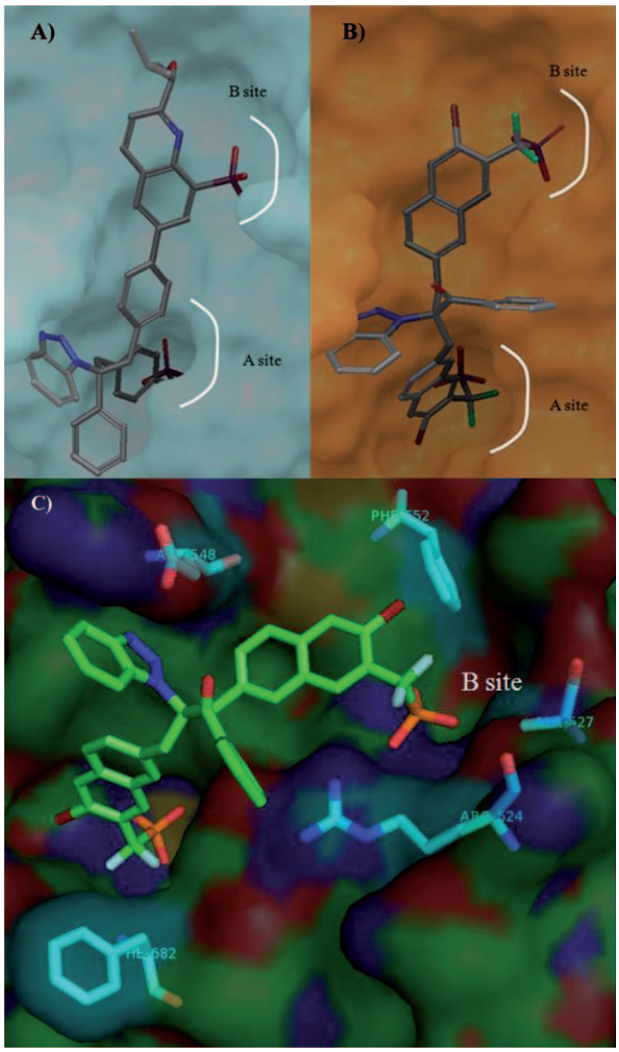


Figure 3. A) X-ray crystal structure of PTP1B in complex with the known inhibitor **2a** (PDB code: 1Q6T). B) Binding pose of **10h** in the PTP1B active site; sites A and B are indicated. Compound **10h** occupies both sites which is essential for PTP1B selectivity over TCPTP. C) Key residues in binding site B surrounding **10h**: upon IFD, Arg524 and Phe682 rotamers change and as a result **10h** docks very well into both sites. Residue numbering is as per current numbering (PDB code: 1Q6T): Arg24 (524), Ala27 (527), Phe52 (552), Arg254 (754), and Met258 (758).

Acknowledgements

We are grateful to the management of Zydus Group for encouragement and are thankful to the analytical department for analytical support.

Keywords: benzotriazoles · inhibitors · metabolic disorders · PTP1B · TCPTP

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[29] Schrödinger Suite 2010, Glide version 5.6, Prime version 2.2, Schrödinger LLC, New York, NY (USA) 2010.

Received: February 9, 2011
Revised: March 17, 2011
Published online on April 19, 2011

