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Design and synthesis of 2-substituted benzoxazoles as novel PTP1B inhibitors

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ABSTRACT

A series of benzoxazole compounds containing oxamic acid were synthesized and screened for the PTP1B inhibition. Compound **31d** showed best biochemical potency (K_i) of 6.7 μM. Structure–activity relationship were explained with the help of molecular modeling approach.

Protein tyrosine phosphatases constitute a large family of signaling enzymes that control several fundamental cellular functions via phosphorylation and dephosphorylation reactions.[1–5] Among PTP's the aberrant expression of Protein tyrosine phosphatase 1B (PTP1B) can contribute to diabetes and obesity.[6–8] Independent studies from two laboratories showed that PTP1B knockout mice exhibit phenotypes of increased insulin sensitivity, improved glucose tolerance and resistance to high fat induced weight gain all without any adverse effects.[9,10] After establishing the PTP1B as an effective target for treatment of both type 2 diabetes and obesity, a lot of efforts were made in the development of potent and selective inhibitors. Designing selective PTP1B inhibitors is a big challenge since PTP1B has highly conserved active site which is found in most of the other PTP's, especially T-cell protein tyrosine phosphatase (TCPTP), a major hurdle in the development of safe and effective PTP1B inhibitors.[11,12] There exists a second non catalytic binding site adjacent to the active site in PTP1B which has been explored for selectivity. PTP1B active site contains phosphotyrosine (pTyr) containing two negative charges at physiological pH. Therefore most of the competitive PTP1B inhibitors have high charge density mimicking pTyr which limits their drug-like properties with limited cell permeability or bioavailability.

Many competitive inhibitors reported contains highly charged anions that mimic the pTyr substrate **1**, such as difluoromethylphosphonates (DFMP) **2**, carboxymethylsalicyclic acids (CMS) **3**, and oxalylaminobenzoic acids (OBA) **4** (Fig. 1). Compound **5**[13] reported earlier has shown good inhibitory potency against PTP1B (K_i = 18 nM) as it had a benzoic acid and a diaryl oxamic acid, a catalytic site binding pharmacophore, but it has exhibited very low cell permeability. Compound **6**[14] reported by the same group had only monoaryl oxamic acid at *para* position exhibited moderate inhibitory (with R = Boc, K_i = 8.8 μM and with R = Ac, K_i = 6.1 μM) activity towards PTP1B. Compounds **7a**[15] and **7b**,[16] are nonpeptidic sulfonamide containing novel (*S*)-isothiazolidinone ((*S*)-IZD) which are pTyr mimetics having IC_{50} of 32 nM and 100 nM respectively.

Based on the above literature data, we designed a series of benzimidazole and benzoxazole molecules. These designs were prioritized based on molecular docking studies in both PTP1B and TCPTP X-ray structures. Molecular docking study was carried out in PDB ID: 2VEU[16] and 1L8K corresponding to PTP1B and TCPTP respectively. These proteins were prepared using protein preparation wizard in the Schrodinger software (Version 2011). 3-D structure of all synthesized compounds was prepared using ligprep[17] module of Schrodinger software (Version 2.5) prior to docking. Docking was carried out in GOLD[18] version 5.1 using very flexible docking methods, which represent high precision docking protocol using GoldScore.

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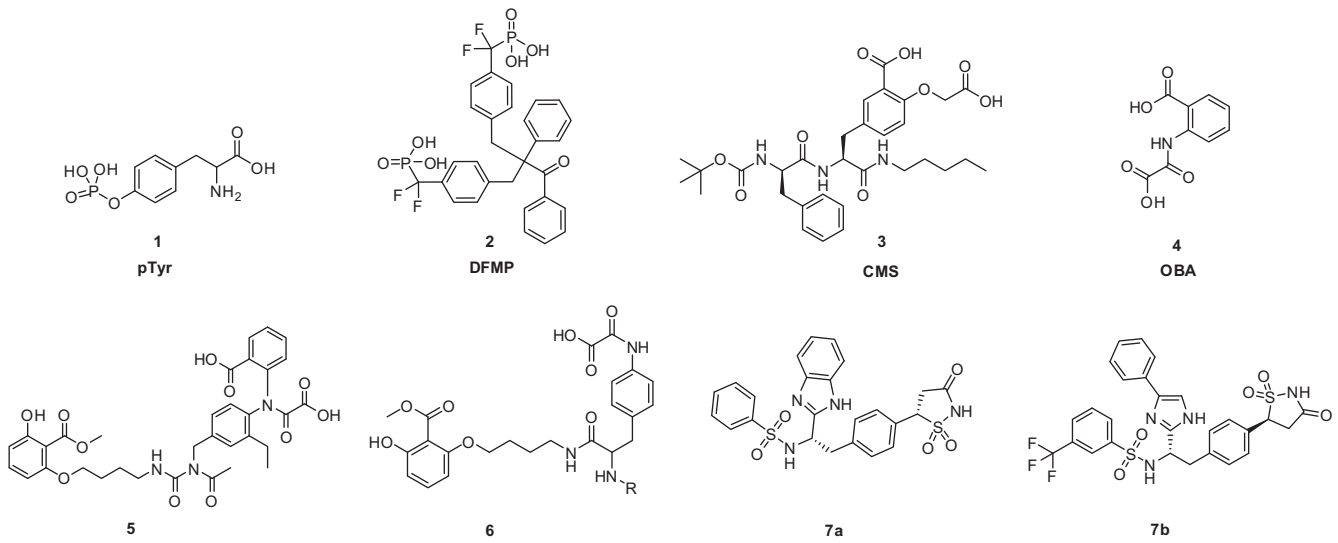
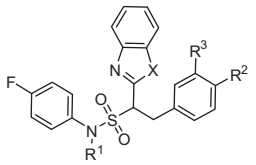


Figure 1. Structures of some known pTyr mimetics and literature known compounds.

Table 1
Inhibitory activities of the synthesized compounds against PTP1B



Entry	X	R^1	R^2	R^3	PTP1B K_i (μM)
16	NH	H	CO_2H	H	>500
20a	NH	H	OCH_2CO_2H	OH	162
19a	NH	H	$NHCOCO_2H$	H	67.5
18c	NH	H	OH	OH	211.5
20b	O	H	OCH_2CO_2H	OH	66.5
19b	O	H	$NHCOCO_2H$	H	35
19c	NH	Me	$NHCOCO_2H$	H	125

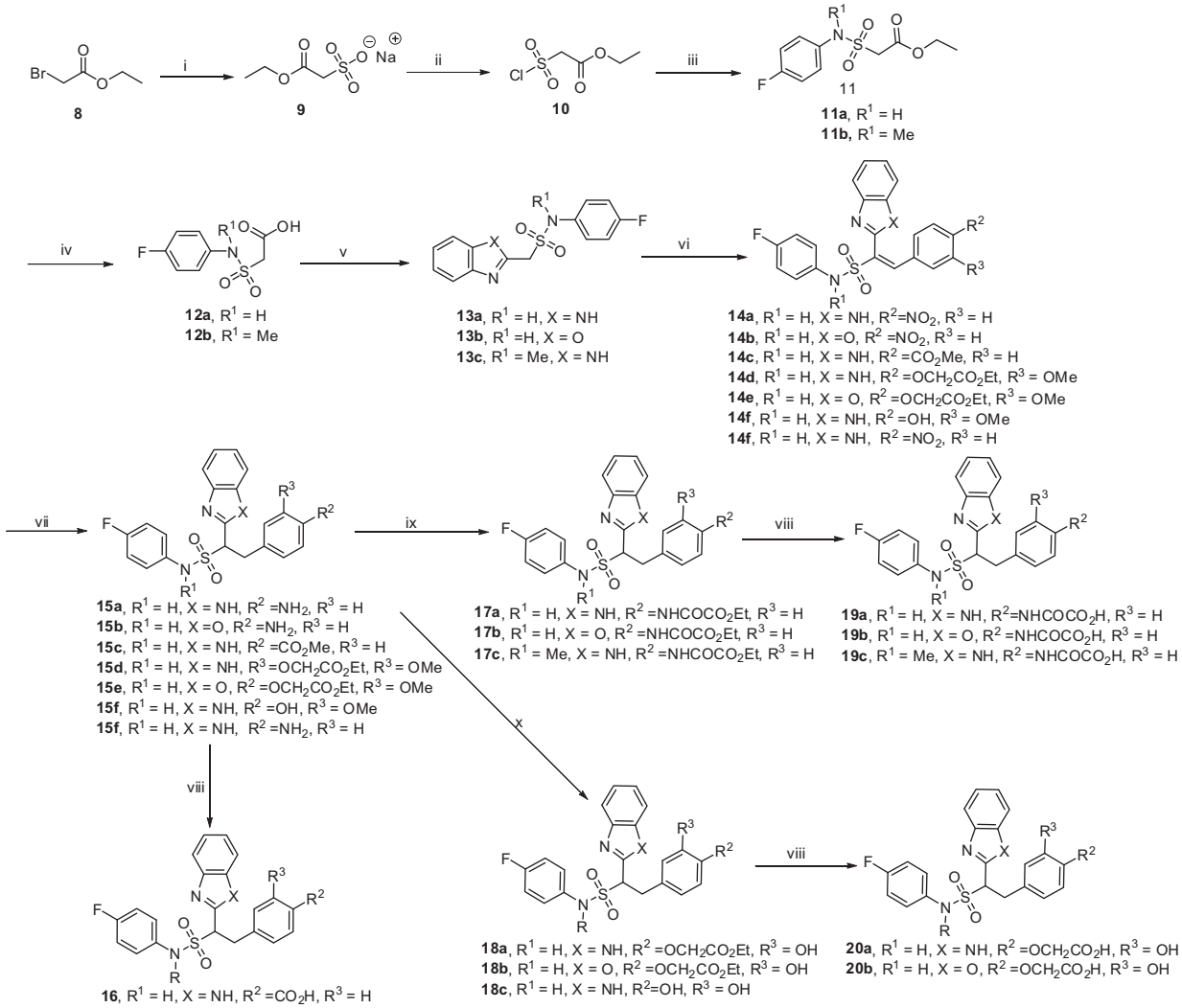
Molecules prioritized based on modeling study were synthesized. Biochemical potency of these compounds was determined in both PTP1B and TCPTP enzymes, results is summarized show in Table 1. Synthesis of benzimidazole and benzoxazole derivatives **16**–**20** is outlined in Scheme 1. Initially benzimidazole and benzoxazole molecules were synthesized using the 4-fluorobenzene sulfonamide as scaffold and by changing the pharmacophore group. Ethyl bromoacetate, **8** was reacted with sodium sulfite and the resulting sodium salt **9** was reacted with phosphorous pentachloride to get the sulfonyl chloride **10**. This sulfonyl chloride was further reacted with 4-fluoroaniline to obtain the sulfonamide, **11a**–**b**.[19] The ethyl ester hydrolyzed to corresponding acids **12a**–**b** using aqueous potassium hydroxide. These carboxylic acids is further reacted with the *O*-phenylene diamine or 2-amino phenol in presence of Lawesson's reagent, neat, in sealed tube at 150 °C to get the corresponding benzimidazoles and benzoxazoles, **13a**–**c**.[20] The active methylene group of these compounds was condensed with substituted benzaldehydes via Knoevenagel condensation using trimethyl silyl chloride and DMF to obtain the olefins, **14a**–**f**.[21] Olefins were reduced with 10% Pd/C in methanol in presence of hydrogen gas at pressure of 15–60 pounds per square inch (psi) to furnish compounds **15a**–**f**. Compounds **15a**–**f** were reacted with different reagents to provide compounds **16**, **18c**, **19a**–**c** and **20a**–**b**.

Biochemical potency of all these compounds was determined for PTP1B. Among the benzimidazole and benzoxazoles synthesized, benzoxazole compounds with oxamic acid containing compounds showed better activity compared to the other compounds. Substitution on the sulfonamide with methyl group reduced potency significantly.

Molecular docking study on compound (**19b**) suggests oxamic acid found to be hydrogen bonded to with key residues like Ser-216, Ala-217, Gly-218, Ile-219, Arg-221 at site A (Fig. 2). These are the same residues that interact with p-Tyr. Thus oxamic acid was found to be a good replacement of highly charged p-Tyr motif. It is evident from modeling study that 4-fluorobenzene ring of the *R*–isomer[26] found to be stabilized by a π-stacking interaction with residue Phe-182. Compound **19b** benzoxazole moiety is positioned at Site-B. Comparing binding model of **19a** and **19b** with respect to benzimidazole and benzoxazole, we could not observe any difference in terms of additional interaction at Site-B. However due to favorable electronegativity of oxygen in benzoxazole compared to benzimidazole nitrogen **19b** shows better potency. Compound **19c** contains benzimidazole and methyl substitution at sulfonamide position showed less PTP1B biochemical potency compared to **19a**. Loss of potency in the case of compound **19c** can be explained based on the fact that methyl incorporation at sulfonamide might destabilize π-stacking interaction with residue Phe-182.

Benzoxazole are preferred at Site-B compared to benzimidazole as observed in compound **19a**. Thus oxamic acids with benzoxazole found to enhance PTP1B biochemical potency so to further analyze the SAR, we thought of modifying the sulfonamide group with different substitutions.

For effecting such a change, we followed similar scheme which is shown in the Scheme 1. As it is explained in the Scheme 2 the sulfonyl chloride, **10** was reacted with *t*-butyl amine in presence of triethyl amine to get the sulfonamide **21**. The ester was hydrolyzed to get the acid, **22** and was reacted with 2-aminophenol in presence of Lawesson's reagent as reported earlier to yield benzoxazole **23**. This was condensed with 4-nitrobenzaldehyde via Knoevenagel condensation, using trimethyl silyl chloride and DMF to furnish the olefin **24**. Both the olefin and nitro groups were reduced using 10% Pd/C under 60 psi hydrogen pressure. The amine **25** was reacted with ethyl oxalyl chloride to form the oxamic acid ethyl ester **26**. Removal of the tertiary group of compound **26** by trifluoroacetic acid furnished the desired primary sulfonamide **28**. Sulfonamide was reacted with alkyl halides to get mono as well as disubstituted products, **30**,[22] which upon hydrolysis furnished oxamic acids, **31**. Similarly sulfonamide **28** was reacted with acid



Scheme 1. Reagents and conditions: (i) Sodium bisulfite, ethanol/H_2O, rt, 16 h; (ii) PCl_5, neat, 100 °C, 4 h; (iii) 4-Fluoroaniline or *N*-methyl 4-fluoro aniline, Et_3N, DCM, 0 °C to rt, 16 h; (iv) KOH, THF, H_2O,rt, 4 h; (v) (a) *O*-Phenylene diamine, or 2-amino phenol, Lawesson's reagent, 150 °C, sealed tube, 2 h; (vi) substituted benzaldehydes, TMSCl, DMF, 135 °C, sealed tube, 4 h; (vii) 10% Pd/C, MeOH, 1–4 Kg H_2, 4–16 h; (viii) KOH, THF/H_2O, rt, 4 h; (ix) Ethyl oxalyl chloride, DMAP, DCM, 0 °C to rt,16 h; (x) BBr_3, DCM, −40 to 0 °C, 1 h.

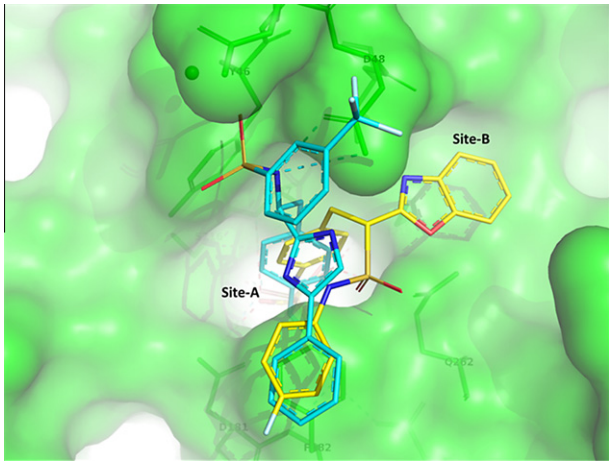
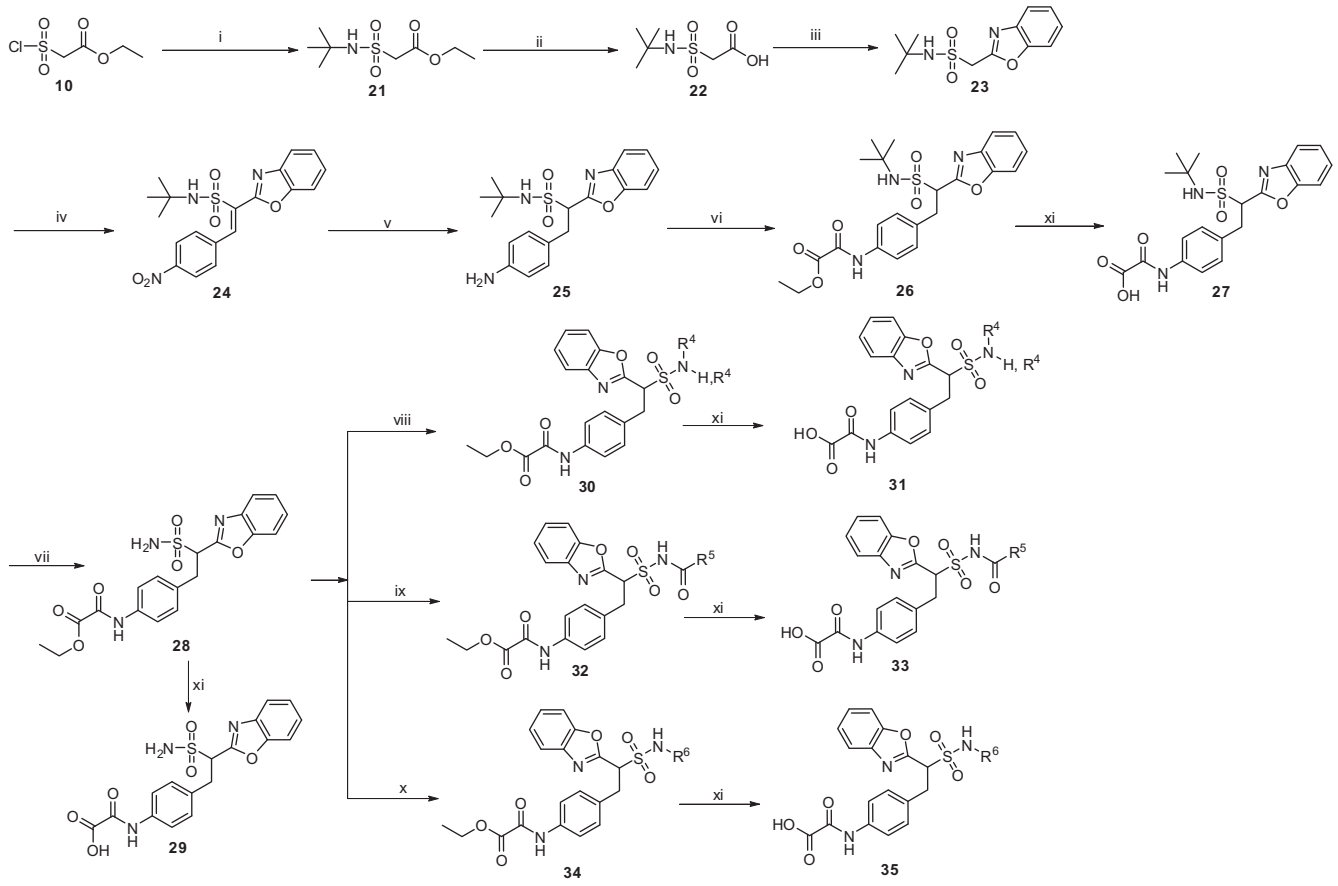


Figure 2. Docking model of compound **19b** (yellow, *R*-isomer) in PTP1B phosphotyrosine binding site. X-ray structure of one of the potent compound[16] from literature (compound **7b** (*S*-isomer), PTP1B IC_{50} = 100 nM, TCPTP IC_{50} = 61 nM) shown in cyan color.

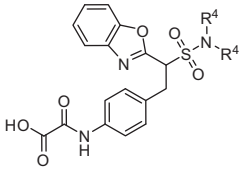
chlorides to give sulfomoyl amides, **32**[23] and upon hydrolysis yielded **33**. Monophenyl sulfonamides, **34** were prepared by Chan–Lam coupling,[24] using copper(ii)acetate and after hydrolysis furnished oxamic acids **35**. For all the synthesized compounds in vitro assay was carried out and the results obtained are shown in Tables 2–4.

All assays were carried out using a standard protocol. The 50 μL reaction was carried out at room temperature in a buffer consisting of 25 mM Tris pH 7.5, 75 mM NaCl, 1 mM DTT and 0.1% BSA. The reaction was started by adding PTP1B protein at 5 nM concentrations. The diluted compounds ranging from 10 mM to 0.1 μM concentration were added to the reaction mixtures in a 96 well plate. The reaction was terminated after 60 min by 1NaOH. The absorbance values of the *p*-nitrophenol were measured at 405 nm using spectrophotometer (spectra MAX190). Enzymatic activity was determined by measuring the absorbance values of 4-nitrophenol with the appropriate correction for the substrate pNPP and the absorbance of the compounds. Assays were carried out keeping the sodium orthovanadate (K_i = 0.67 μM)[25] as reference compound. The inhibition (K_i) values were obtained by fitting the data using non linear regression hyperbolic fit to Michaelis–Menten



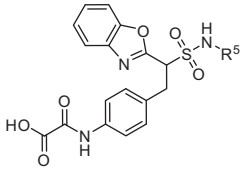
Scheme 2. Reagents and conditions: (i) *t*-Butyl amine, Et_3N, DCM, 0 °C to rt; (ii) KOH, THF, H_2O; (iii) 2-Amino phenol, Lawesson's reagent, 150 °C, 2 h, sealed tube; (iv) 4-Nitro benzaldehyde, TMSCl, DMF, 135 °C, 4 h, sealed tube; (v) Pd/C, MeOH, 4 Kg H_2, rt, 16 h; (vi) Ethyl oxalyl chloride, DMAP, DCM, 0 °C to rt, 16 h; (vii) TFA, 50 °C, 6 h; (viii) R^4Br, K_2CO_3, DMF, rt, 16h; (ix) R^5COCl, K_2CO_3, acetone, reflux, 4 h; (x) $R^6B(OH)_2$, $Cu(OAc)_2$, pyridine, DCM, rt, 48 h; (xi) KOH, THF/H_2O, rt, 4h.

Table 2
Inhibitory activities of the alkylated compounds against PTP1B and TCPTP



Entry	R^4	R^4	PTP1B K_i (μM)	TCPTP K_i (μM)
29	H	H	331	136.5
27	*t*-Bu	H	228	135
31a	*n*-Bu	H	82.4	33.4
31b	CH_2CH_2(4-Cl Ph)	H	41	—
31c	4-FBn	4-Fbn	10	—
31d	4-ClBn	4-ClBn	6.7	3.7
31e	2-CF_3Bn	2-CF_3Bn	6.8	—
31f	3-CF_3Bn	3-CF_3Bn	35	21
31g	4-CF_3Bn	4-CF_3Bn	7.7	12.5
31h	4-OMeBn	4-OMeBn	14	18.9
31i	4-OCF_3Bn	4-OCF_3Bn	16.8	17.2
31j	4-*t*-BuBn	4-*t*-BuBn	14.9	6.4
31k	CH_2CO_2H	CH_2CO_2H	235	22

Table 3
Inhibitory activities of the aryl compounds against PTP1B and TCPTP



Entry	R^5	PTP1B K_i (μM)	TCPTP K_i (μM)
35a	4-MePh	62	9.2
35b	4-CF_3Ph	23.6	8
35c	4-OMePh	68.8	134
35d	4-Oi-PrPh	92	101

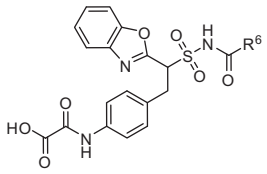
enzyme kinetic model. These molecules showed the moderate activity against the PTP1B.

Compound **28**, without any substitution on the sulfonamide has shown very less inhibition towards PTP1B. Mono substitution of the sulfonamide with tertiary butyl group (**27**) failed to improve potency. Introducing the *n*-butyl (**31a**) and 4-chlorophenethyl groups (**31b**) on the sulfonamide slightly improved the activity.

Direct substitution of the phenyl ring to the sulfonamide provided no major increase in activity. Among the 4-fluoro (**19b**), 4-methyl (**35a**), 4-trifluoromethyl (**35b**), 4-methoxy (**35c**) and 4-isopropoxy (**35d**) substituted phenyl rings, 4-trifluoromethyl substituted (**35b**) product showed marginally better inhibition.

Di-substitution of the benzyl groups on sulfonamide NH_2 has shown better potency in both PTP1B and TCPTP compared to the mono substituted compounds. Molecular docking study in PTP1B suggests compound **31d** oxamic acid found to hydrogen bonded to with key residues like Ser-216, Ala-217, Gly-218, Ile-219,

Table 4
Inhibitory activities of the acyl compounds against PTP1B and TCPTP



Entry	R^6	PTP1B K_i (μM)	TCPTP K_i (μM)
33a	Ac	63	86.5
33b	Ph	125	256
33c	4-ClPh	82.4	13.1

Arg-221 at site A (Fig. 3). 4-chlorobenzene ring of the *R*-isomer[26] found to be stabilized by a π-stacking interaction with residue Phe-182. Similar bioactive conformation was seen in compound **7b**[16] where phenyl ring attached to imidazole ring has found to have π-staking interaction with same residue. It is possible that residue Tyr-46 may facilitate similar stacking interaction in Site-A. Optimum positioning of the benzylic groups in Site-A optimizes the π-stacking interaction with both residues Phe-182 and Tyr-46 resulting in better potency. Loss of biochemical potency for **29** might be attributed to lack of any π-stacking interaction with Phe-182. With various halo substituted benzyl ring at this position has resulted in better potency due to their highly electronegative effect. Similarly 2-trifluoromethyl (**31e**) and 4-trifluoromethyl (**31g**) substituted benzyl compounds shown reasonably good potency but with the 3-trifluoromethyl substituted compound (**31f**) there was fourfold decrease in PTP1B biochemical potency. Compound **31k** with disubstituted polar group acetic acid lost potency due to the loss of the π-stacking interaction. In similar direction, compounds **33a**, **33b** and **33c** with acylsulfomoyl groups on the sulfonamide NH_2 showed less potency compared to the dibenzyl sulfonamides because of the absence of any stacking interactions with residues Phe-182 and Tyr-46.

Docking model of compounds **31d** and **29** in TCPTP suggests these compounds are confided to Site-A (Fig. 4). Oxamic acid of these compounds found to be hydrogen bonded with key residues like Ser-217, Ala-218, Gly-219, Ile-220, Arg-222 at Site-A in TCPTP. Positioning of oxamic acid along with benzoxazole at Site-A for compound **31d** and **29** found to be similar. However additional occupancy of benzylic groups at Site-A in the case of compound **31d** provides better TCPTP potency compared to other compounds.

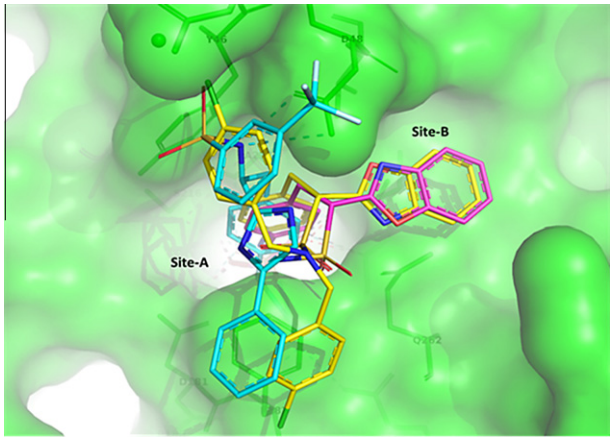


Figure 3. Docking model of compound **31d** (yellow, *R*-isomer) and **29** (magenta, *R*-isomer) shown in PTP1B phosphotyrosine binding site. X-ray structure of one of the potent compound[16] from literature (compound **7b**, PTP1B IC_{50} = 100 nM, TCPTP IC_{50} = 61 nM) shown in cyan color.

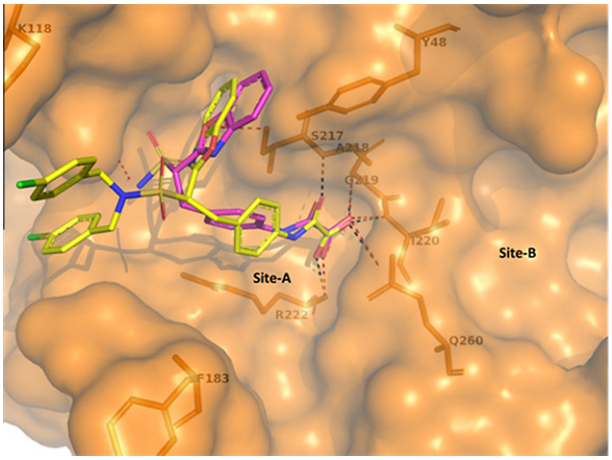


Figure 4. Docking model of compound **31d** (yellow, *R*-isomer) and **29** (magenta, *S*-isomer) is shown in TCPTP phosphotyrosine binding site.

In summary, two series of compounds containing benzimidazole and benzoxazole were prioritized and synthesized, based on molecular modeling studies. Novel benzoxazole series with di-substitution on the sulfonamide showed the better inhibition toward PTP1B due to the π-stacking interaction with the residue Phe-182. Compound **31d** found to be the best among these di-substituted sulfonamide compounds reported with K_i value of 6.7 μM, due to the electronegative effect of *para* chloro substitution. Further investigation of these derivatives with change of pharmacophore and in vitro assays of the benzothiazole series are in progress and will be reported in due course.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.109. These data include MOL files and InChiKeys of the most important compounds described in this article.

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26. Supplementary data includes following documents: NMR data, experimental procedures, docking results of each enantiomers mentioned in the main text, compounds mol files.